STUDIES ON THE PATHOGENESIS OF ACUTE GALLSTONE PANCREATITIS

CHRISTOPHER PAUL ARMSTRONG

MBChB 1978

FRCS Ed.

FRCS Eng.

Degree of Doctor of Medicine

University of Edinburgh

1985

CONTENTS

PART 3 - THE BILIARY TRACT IN PATIENTS WITH ACUTE GALLSTONE PANCREATITIS

ABBREVIATIONS

AGP	-	acute gallstone pancreatitis.
AHP	-	acute haemorrhagic pancreatitis.
BPD	-	bile pancreatic duct.
CCK	-	cholecystokinin.
ERCP	-	endoscopic retrograde cholangiopancreatography.
CA	-	cholic acid.
PGWR	-	pancreatic gland weight ratio.
WC	-	water content of gland.
SA	-	serum amylase.
PFA	-	peritoneal fluid amylase.
GA	-	gland amylase.
SO	-	sphincter of Oddi.
PLA_2	-	phospholipase A_2.
DOC	-	deoxycholic acid.
GDC	-	glycodeoxycholic acid.
TC	-	taurocholic acid.
CA	-	cholic acid
CDCA	-	chenodeoxycholic acid.
E. Coli	-	Escherichia Coli.
PMB	-	pancreatic duct mucosal barrier.
u/s	-	ultrastructure.
SPS	-	standard perfusate solution.
Ca^{2+}	-	calcium ion.
HCO_3^-	-	bicarbonate ion.
Cl^-	-	chloride ion.
[ ]	-	ion concentration.
pD	-	transductal potential difference.
PG	-	prostaglandin.
mM	-	millimole.
µmol	-	micromole.

µl	-	microlitre.
ml	-	millilitre.
$J.Cl^-$	-	flux of chloride ions. (µmol/cm/hr)
$J.HCO_3^-$	-	flux of bicarbonate ions. (µmol/cm/hr)
$\Delta J.Cl^-$	-	change in flux of chloride ions.
$\Delta J.HCO_3^-$	-	change in flux of bicarbonate ions.
ΔpD	-	change in potential difference.
PDR	-	pancreatic duct reflux.

LIST OF FIGURES

13. serum amylase vs pressure.

14. peritoneal fluid amylase vs pressure.

15. Histology of rat pancreas.

    A. normal gland (x 140)

    B. normal gland (x 120)

    C. severe oedema (x 120)

    D. severe oedema (x 150)

16. Histology score for 5 minutes occlusion.

17. Histology score for 60 minutes occlusion.

18. Histological appearance of duct rupture (x 200).

19.A+B Diagrammatic representation of extravasation.

20. Bile and infection vs.

    A. PGWR

    B. SA

    C. PFA

    D. Histology score.

21. Histological appearances of

    A. moderate pancreatitis (x 150)

    B. severe haemorrhagic pancreatitis (x 150)

22. Histological appearance of

    A. normal duct (x 300)

    B. ducts filled with acute inflammatory cells (x 140)

    C. duct-moderate inflammation (x 250)

    D. duct-severe inflammation (x 200)

    E. duct-severe inflammation (x 250)

23. Filtered bile vs.

    A. PGWR

    B. SA

23. C. PFA

D. Histology score

24. Diagrammatic representation of PMB.

25. Electron microscopy.

A. normal duct (x 7500)

B. normal duct (x 7500)

26. Apical complex in rat BPD (x 75,000)

27. Stages in infusion technique.

A. ligatures around distal and proximal BPD

B. cannulation of proximal BPD

C. cannulation of distal and proximal BPD

D. perfusion of duct.

28. Experimental preparation.

29. Effluent anionic concentration vs. flow rate.

30. Anionic flux.

31. Volume vs ^{14}C PEG

32. Transductal pD vs. $[HCO_3^-]$.

33. Bile, infection and pressure vs.

A. chloride flux

B. bicarbonate flux

C. potential difference

34. Electron microscopy of duct

A. low pressure (x 11,250)

B. high pressure (x 11,250)

C. sterile bile, L.P. (x 7500)

D. sterile bile, H.P. (x 11,250)

E. infected bile, L.P. (x 7500)

F. infected bile, H.P. (x 11,250)

34. G. disruption duct wall (x 7500)

H. underlying oedema (x 11,250)

35. Bile salt vs.

A. chloride flux

B. bicarbonate flux

C. potential difference

36. E.M. after bile salts (x 11,250).

37. PLA_2/lysolecithin vs. ($\pm$ lecithin)

A. chloride flux

B. bicarbonate flux

C. potential difference

38. E.M. of duct after PLA_2/lysolecithin.

A. vacuolation (x 11.250)

B. vacuolation (x 11.250)

C. epithelial elevation (x 4250)

D. epithelial disruption and cell death (x 11,250)

39. GDC and lecithin vs.

A. chloride flux

B. bicarbonate flux

C. potential difference

40. cytoprotection of duct epithelium

A. EM - lysolecithin alone (x 5000)

B. EM - lysolecithin + lecithin (x 11,250)

C. EM - GDC + lecithin (x 11.250)

41. Prostaglandin E_2 and bile salts vs.

A. chloride flux

B. bicarbonate flux

C. potential difference

42. Patient studies; measurements taken.

43. Number of gallbladder calculi.

44. Size of smallest calculi.

45. Size of cystic duct.

46. Size of common bile duct.

47. Examples of PDR in patients with gallstone pancreatitis.

    A. whole pancreatic duct

    B. stones in common bile duct and at ampulla

    C. loop in pancreatic duct

    D. associated duodenal diverticulum

LIST OF TABLES

19. Phospholipase A_2/lysolecithin peritoneal fluid amylase.

20. Phospholipase A_2/lysolecithin Histology score.

21. Intraductal pressure at varying rates of perfusion.

22. Percentage recovery of ^{14}C PEG following BPD perfusion at varying flow rates.

23. Bile and infection vs. anionic flux. Low pressure.

24. Bile and infection vs. anionic flux. High pressure.

25. Bile salts vs. anionic flux.

26. PLA_2/lysolecithin vs. anionic flux.

27. The effect of lecithin on GDC induced damage.

28. The effect of prostaglandin E_2 on GDC induced damage.

29. The effect of lecithin on PLA_2/lysolecithin induced damage.

30. Significant differences between patients with AGP and controls.

ACKNOWLEDGEMENTS

The work described in this thesis was performed in the Department of Surgical Gastroenterology, Manchester Royal Infirmary and the University of Manchester Medical School. This project was funded by a "Thomas Markland" research scholarship awarded by the British Digestive Foundation and I wish to thank both the Markland family and the BDF for their generous support.

I am grateful to Mr Vin. Taylor, Consultant Surgeon, without whom this thesis would not have come to fruition. His enthusiasm, foresight, interest and kind help will always be an inspiration to my research. I am also indebted to Mr Bruce Torrance, Consultant Surgeon, for his direction, support and advice throughout the period of research.

Further thanks are extended to Professor R. M. Case and staff in the secretion laboratory, Department of Physiology for their help and advice. Their facilities and technical expertise have enabled the thesis to reach its present form.

I also acknowledge the advice and help of the Department of Anatomy in the preparation and interpretation of histology and electron microscopy.

The last part of this thesis owes much to the enthusiasm and help of Mrs Julie Jeacock and Mr Sam Lucas, Department of Computation. I wish to thank both Jackie and Stephanie in S3 Records for their kind help.

Last but not least I am grateful to Elaine McDermott for typing the thesis and to Jane for her patience and support throughout this period.

DECLARATION

This thesis has been composed entirely by the author. All procedures described have been carried out personally, with assistance in the preparation of electron microscopy.

ABSTRACT

This study has evaluated factors thought to be important in the pathogenesis of acute gallstone pancreatitis (AGP) by means of (i) experimental rat preparations and (ii) a prospective study of a large group of patients with gallstone related disease.

Retrograde injection into the rat bile-pancreatic duct was employed. Pressure and volume of injectate were critical determinants of pancreatic damage and these were subsequently strictly controlled to be within physiological range. Infusion of human bile at low pressure gave moderate pancreatic damage whereas infected bile was associated with a high incidence of acute haemorrhagic pancreatitis. Bile from patients with AGP was more toxic than bile from controls. Trypsin, enterokinase and bile salts individually produced mild pancreatic damage whereas in combination severe damage resulted. Active phospholipase A_2 (PLA_2) and lysolecithin gave moderate to severe pancreatitis.

Stability of the "pancreatic duct mucosal barrier" (PMB) was assessed by perfusing the rat bile pancreatic duct and measuring ionic permeability, transductal potential difference and ultrastructural appearances of the duct wall. This preparation was fully characterized and found to act in a reproducible manner between animals. Bile, especially when infected or under pressure, was extremely noxious to the duct and produced marked epithelial disruption and striking changes in permeability. Bile salts damaged the PMB; the damage being reversible with low concentrations and irreversible with high

concentrations of the bile salt. Active PLA_2 and lysolecithin produced equally severe damage to the PMB. Cytoprotection of the duct epithelium against bile salt and PLA_2/lysolecithin induced damage was afforded by both lecithin and prostaglandin E_2.

Features of the biliary tract (clinical and operative) were prospectively studied in a series of 52 patients with AGP and compared with 610 controls. Patients who developed pancreatitis were more often male, had multiple small gallbladder calculi and a wider cystic duct than controls. The common bile duct diameter was independent of the presence of stones in AGP patients and the time of operation after the onset of symptoms was a critical determinant of biliary pathology. Pancreatic duct reflux was significantly more common in patients with AGP and reflux occurred into a wider pancreatic duct, at a greater angle and via a longer common channel in such patients. These features are highly suggestive of gallstone migration being an essential mechanical initiator of AGP.

Patients who develop AGP have a biliary tract that predisposes to gallstone migration and pancreatic duct reflux. Furthermore these patients may have an imbalance in attack and defence forces within the pancreatic duct which promotes extravasation and subsequent pancreatic inflammatory sequelae.

INTRODUCTION

The clinical problem of acute pancreatitis was known long before Claude Bernard (1850) began to investigate the association of the biliary tract with pancreatitis by injecting a mixture of bile and olive oil into the pancreatic duct to produce acute haemorrhagic pancreatitis (Galen, quoted by Fitzgerald 1980). The problem was further explored by Prince (1882) who noted an association between cholelithiasis and pancreatic haemorrhage and suggested that pancreatic injury might be "dependent upon the pancreatic duct being obstructed by a gallstone becoming impacted in the duct common to it and the ductus communis choledochus". Nicholas Senn (1886) comprehensively reviewed his experimental work on the pancreas. He reported three fatal cases of haemorrhagic gangrenous pancreatitis; the milder forms of acute pancreatitis were never diagnosed in Senn's day. It was Reginald Fitz, however, who in 1889 specified signs and symptoms and firmly established the disease entity with its gangrenous, haemorrhagic and suppurative aspects. His work, published in the Boston Medical and Surgical Journal, concluded that acute inflammation of the pancreas was both a definite clinical entity and one that was much more frequent than generally believed.

Although Bernard (1850) had explored the association of the biliary tract with pancreatitis and Lancereaux (Gambill 1973) in 1899 had suggested that reflux of bile might be the cause of pancreatitis, it was not until Opie's work in 1901 that the association of gallstones and pancreatitis was proven. Opie, a pathologist at Johns Hopkins Hospital, performed an autopsy on a patient with fatal acute haemorrhagic pancreatitis: his description of the findings is as follows.

"The diverticulum of Vater was 10 mm in length. Lodged at its apex blocking its duodenal orifice was a small calculus only 3 mm in diameter, but too small to pass the narrow opening. Though it occluded the duodenal orifice of the diverticulum it was so small that the orifices of the common bile duct and pancreatic duct were unobstructed. The two ducts were therefore, converted into a continuous closed channel from which it was not possible for either bile or pancreatic juice to escape". This observation prompted his classic "common channel thory" (Opie 1903, 1909) which dominated the thinking of numerous scholars in the field for several decades.

Since Opie's original description there has been much controversy over the role of gallstones in producing acute pancreatitis. Several authors (Dragstedt 1934, Ivy 1952, Grossman 1959, Banks 1971) emphasized the low incidence of finding a stone at the ampulla in patients with haemorrhagic pancreatitis. It was these observations, and the finding of a common channel in only 50-70% of patients, that led McCutcheon (1968) to propose duodenal reflux as being more important than bile reflux in the pathogenesis of acute gallstone pancreatitis. Recently, however, Acosta (1974) and Kelly (1976) have offered an explanation for the association of gallstones with acute pancreatitis. These authors have developed the "gallstone migration" theory to explain the low incidence of ampullary stones found at surgery. Indeed it is now well established that gallstone migration, with its attendent bile (fig. 1) or duodenal reflux (fig. 2), is important in the pathogenesis of acute gallstone pancreatitis (Acosta 1978, Kelly 1982).

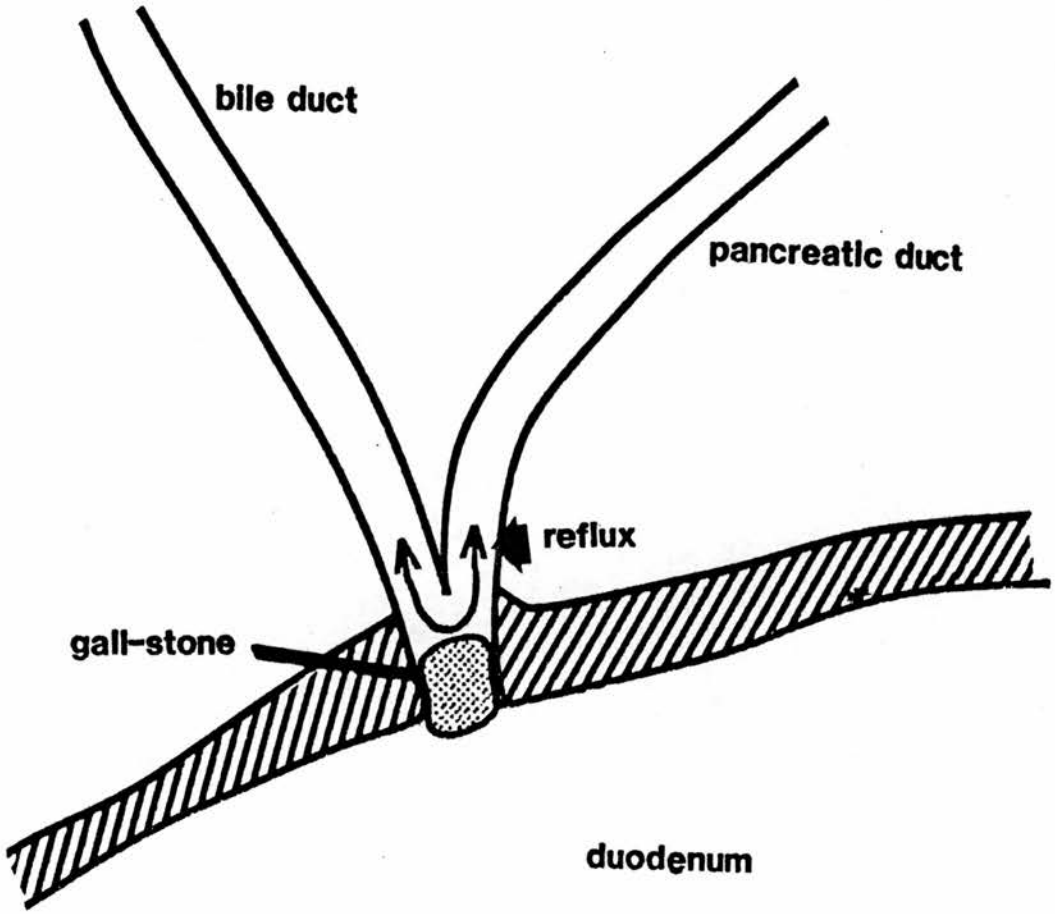


Fig. 1 - Migrating gallstone; Bile reflux.

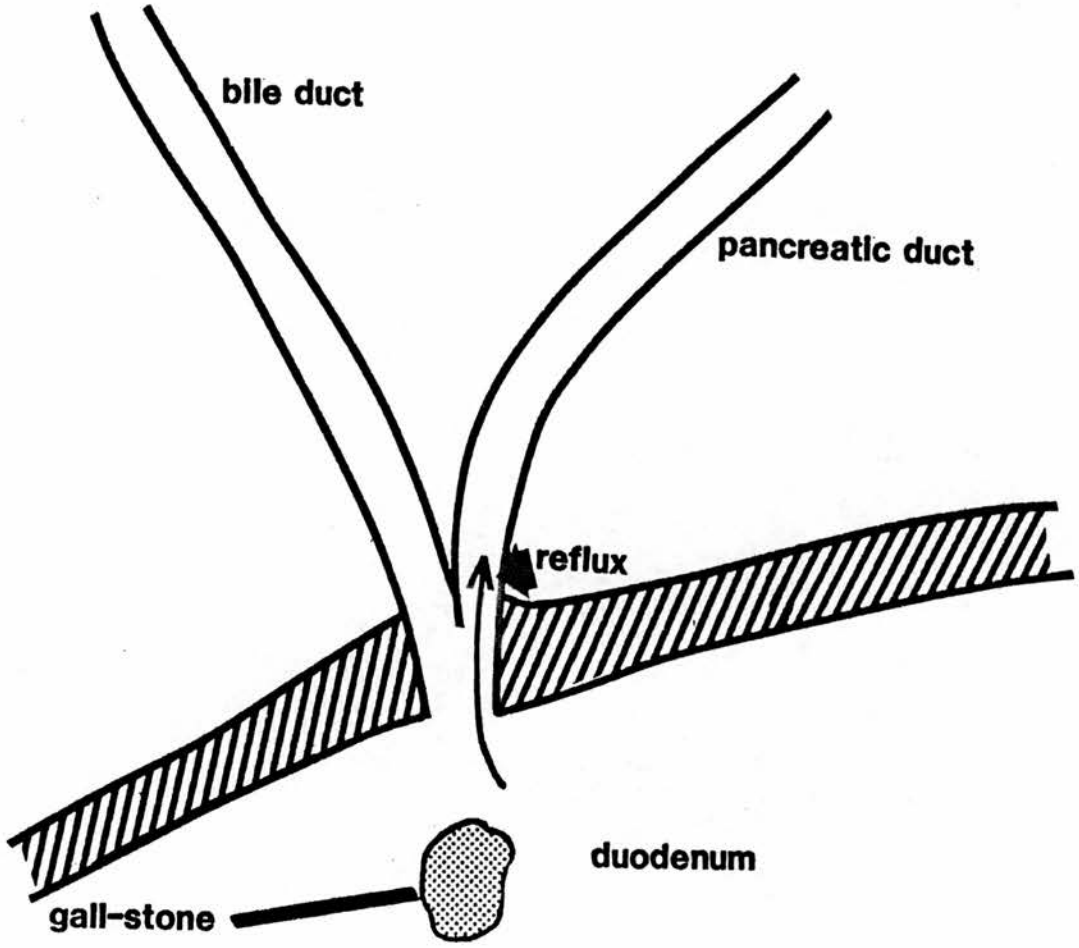


Fig. 2 Migrating gallstone; Duodenal reflux.

ACUTE GALLSTONE PANCREATITIS

The predominant aetiological factors in the genesis of acute pancreatitis are gallstones and alcohol; together accounting for over 75% of the total. The association of gallstones with acute pancreatitis is well established. Moreover, the very low incidence of recurrent attacks of acute pancreatitis following removal of all gallstones attests to their importance in initiating pancreatic inflammation. The incidence of gallstones in acute pancreatitis varies considerably in different parts of the world. In Europe and North America, the percentage of patients in whom gallstones are responsible for the acute pancreatitis varies from 40 to 60%. A summary of major series is given as follows:

	No. of cases	% due to gallstones
Ivy (1952)	667	55%
Howard and Jordan (1960)	?	45-55%
White (1968)	733	54%
De Bolla (1984)	897	57%
Hermon-Taylor (1977)	?	45-57%
Trapnell (1975)	591	54%
Durr (1979)	3836	41%
Total	≏ 7000	40-60%

The close correlation between acute pancreatitis and biliary tract disease is further evidenced from large autopsy series. Bell (1958) studied 10,387 unselected autopsies of whom 1874 patients (18%) had gallstones. In contrast of 179 patients with pancreatitis 100 (56.7%) had gallstones.

Stones in the common duct are more dangerous than stones in the gallbladder. In the series of Kozoll and colleagues (1959) the incidence of acute pancreatitis was 13% in 146 patients with choledocholithiasis and 3.6% in 634 patients with cholecystolithiasis. Because of the higher frequency of cholecystolithiasis, most patients with gallstone pancreatitis have stones in the gallbladder and not in the common duct. In their review of the literature, Goebell and Hotz (1979) found 1450 cases of gallstone pancreatitis in whom data on the localization of the stones were available. The exact pathology was stones in the gallbladder only 72%, stones in the common bile duct 20%, stones impacted at the ampulla 2% and an inflammed gallbladder without stones in 6%.

Patients with gallstones tend to be older than those with alcohol associated disease. The overall mortality in acute pancreatitis is 10-15%, gallstone pancreatitis being perhaps more dangerous than the alcoholic variety (Carter 1983a,b, Trapnell 1975, Mero 1982, De Bolla 1984, Mayer 1984). Jacobs and associates (1977) have reported mortality rates in acute pancreatitis as gallstone pancreatitis 10%, alcohol 7-8% and idiopathic pancreatitis at 17%. The mortality of haemorrhagic pancreatitis is far greater than the oedematous form (50% vs 3-5%) (Banks 1971) and why some patients develop the haemorrhagic form of the disease as opposed to the oedematous variety remains unclear. There is no evidence that improved standards of care in recent years have significantly reduced the mortality from gallstone pancreatitis.

Aetiology of Acute Gallstone Pancreatitis

There is a large and highly controversial literature on the subject of

acute pancreatitis. Many of the arguments derive from conflicting experimental results, the extrapolation of which to the human clinical situation has usually been speculative. The initiation of acute gallstone pancreatitis forms the basis of three popular theses:-

(i) bile reflux into the pancreatic duct,

(ii) duodenal reflux into the pancreatic duct.

(iii) obstruction of the pancreatic duct with associated hypersecretion.

The relationship of gallstones to pancreatitis in man suggests that the theories of bile and duodenal reflux are the most likely explanations, with reflux being the result of stone passage through the ampulla of Vater.

The temporal sequence of acute pancreatitis might therefore be:

1. presence of gallstones.
2. gallstone migration.
3. reflux of bile and/or duodenal juice.
4. pancreatic duct damage.
5. initiation of inflammation.
6. acute pancreatitis.

Initiation of pancreatic damage is perhaps the least understood event in the genesis of acute gallstone pancreatitis. Several factors need to be reviewed in an attempt to clarify the situation. These factors are listed below and are explained in depth in the succeeding chapters:

- the anatomy of ampulla of Vater.
- pancreatic, duodenal and biliary pressures.

- bile, including bile salts, lecithin, infection and other biochemical substances.
- duodenal juice e.g. bile salts, enterokinase, lysolecithin.
- infection from bile and the duodenum.
- abnormalities in the pancreatic juice.
- enzymes, e.g. trypsin, phospholipase A_2, elastase.
- vascular abnormalities.
- a lymphatic component.
- pancreatic duct resistance.

It has been the aim of this study to evaluate the relative importance of these factors in the pathogenesis of acute gallstone pancreatitis.

Experimental models of Acute pancreatitis

Acute pancreatitis has been simulated in experimental animals for many years. The animals used have varied from dogs, cats, monkeys, pigs to rats and mice. Though dogs and other large animals are useful and reliable models for the induction of acute pancreatitis, the cost of these animals makes large scale investigation prohibitive. It is not surprising, therefore, that recent attention has been given to the smaller, cheaper animals. The rat is an economical and easily maintained laboratory animal. Its cost allows large numbers of experiments to be performed, thus statistically validating or refuting experimental results. It is large enough to allow difficult technical procedures to be performed on it, whilst remaining easy and inexpensive to breed and feed. It was for these reasons that the rat was used as the experimental animal in the present study. A variety of experimental models for the induction of acute pancreatitis have been employed over the years. These are summarized below with their respective advantages and disadvantages. A fuller description of the more important procedures is described at length later in this thesis.

(1) Cannulation of and injection into the pancreatic duct.

This has been employed in large animals where the pancreatic duct is easily cannulated and consists of one or two single channels. A variety of substances have been injected into the duct to produce pancreatic damage, for example, bile, bile salts and enzymes such as trypsin and elastase (Elliott 1957, 1971, Anderson 1958, 1969). This method consistently produces acute pancreatitis but the volumes and pressures employed have been so variable and uncontrolled as to forego

meaningful results. Moreover, only relatively large animals such as the cat and dog lend themselves to direct injection into the pancreatic duct.

(2) Cannulation of the rat bile-pancreatic duct and injection.

The anatomy of the biliary tract in the rat makes direct cannulation of the pancreatic duct impossible. In view of this the bile-pancreatic duct has been cannulated, the bile duct occluded and injection commenced into the pancreas (see later). Several substances have been injected into the ductal system including bile salts, enterokinase, lysolecithin and phospholipase A_2 (Donohue 1984, Lankisch 1979, 1983). This method has much to recommend it as the injection can be standardized, the cannula removed at the end of infusion and the biliary and pancreatic anatomy reconstituted. Unfortunately however, the volume and pressure of infusate are far from standardized making a valid comparison between the variously reported experimental results impossible.

(3) Ligation of the common bile/pancreatic duct.

Ligation of the pancreatic duct alone will produce oedema only. When the two ducts are ligated, bile and pancreatic juice undergo admixing and reflux into the pancreatic duct occurs (Wanke 1970, Block 1955). Unfortunately the degree of pancreatic damage is very variable and not all animals develop acute pancreatitis.

(4) Parenchymal injury.

Direct injection of bile, bile salt or activated pancreatic enzymes into the pancreatic parencyma is a popular and easy way of producing acute pancreatitis (Bawnik 1974, Keith 1958).

The relevance of this method to the human situation is debateable.

(5) Duodenal Closed loop

Obstruction of the duodenum on both sides of the ampulla of Vater allows a build up of intraduodenal pressure. This leads to reflux of duodenal contents into the pancreas under pressure. The duodenal contents consist of a mixture of bile, pancreatic juice, activated enzymes, micro-organisms and duodenal secretions which are highly toxic to the pancreas. This closed loop preparation was initially described by Pfeffer and co-workers in 1957 using a canine preparation and has been referred to as the "Pfeffer loop". Recently several workers have described similar preparations in rats (Nevalanien 1975, Rao 1981, Ferrie 1978, Brackett 1983).

A further development was described by Chetty and associates (1980) who injected infected bile into the closed loop under pressure and produced a lethal form of pancreatitis. Questions have recently been raised as to whether death occurs from septicaemia and duodenal necrosis developing after injection of infected bile. Orda and colleagues (1980) introduced a bile salt/ trypsin mixture into the duodenal loop and this produced a severe form of pancreatitis.

These models of acute pancreatitis have the advantage of consistently producing pancreatic inflammation. However, the method involves duodenal trauma and produces a situation which appears unlike that seen in man.

(6) Caerulein pancreatitis.

Acute pancreatic stimulation with supramaximal doses of the synthetic pancreozymin analogue, caerulein, produces acute interstitial pancreatitis (Lampel 1977, Adler 1982). Adler (1982) has shown that caerulein alters membrane fusion and acinar cells discharge their content into the interstitial space. The pancreatitis produced is mostly oedematous in nature with a small inflammatory infiltrate but no haemorrhagic changes are observed.

The need to give supramaximal doses of caerulein and the type of pancreatic inflammation produced negate the use of this model in the investigation of AGP.

(7) Metabolic pancreatitis (Farber 1950)

This method, initially reported in the 1950's, was improved recently by Rao and colleagues (1976) who described a model of acute pancreatitis in mice. The condition was induced by feeding a choline deficient diet supplemented with ethionine, and invariably produced a severe fatal pancreatitis in 3 to 5 days. The nature of the choline deficient, ethionine supplemented diet-induced lesion which eventually progresses to haemorrhagic necrosis of the pancreas has not yet been elucidated. This model produces reliable, reproducible pancreatitis which resembles the human disease, without laparotomy and has thus become popular with several recent investigators (Standfield 1983, Coelle 1983). The relevance of this method to acute gallstone pancreatitis is unclear as the method itself

induces widespread systemic metabolic changes.

The above are popular methods currently in use for inducing experimental acute pancreatitis. Other preparations are uncommonly used and their scope is broadly encompassed in the above methods. The experimental models described are highly invasive and the severity of the ensuing pancreatitis has been difficult to control. As a consequence, relatively little useful information has been gained using these models (Steer 1984). Indeed of the models described only methods (1) and (2) can be recommended. If experimentation is to be valid and the results are to be extrapolated to the human situation it is vital that we take cognisance of Elliott's (1971) postulates which state that during injection into the pancreas control should be made of (a) volume of fluid, (b) pressure of injection, (c) fluid constituents and (d) time of injection. The methods described to date have failed to follow these basic postulates and the results obtained cannot therefore be compared or accurately evaluated.

Dissatisfaction with these experimental methods prompted me to develop a standardized physiological preparation for the study of acute gallstone pancreatitis.

PART 1

EXPERIMENTAL ACUTE PANCREATITIS - FACTORS AFFECTING ITS PATHOGENESIS

CHAPTER II

MATERIALS AND METHODS

Animals

Male inbred Sprague-Dawley rats were used for all experiments. The animals weighed 250-350 g and each was carefully weighed before surgery. All animals were fasted for 12 hours overnight before operative intervention.

Anatomy of rat pancreas

The eating habits of common laboratory animals differ considerably. We can divide them into intermittent feeders, which tend to be carnivorous (e.g. cat, dog) and continual feeders, which tend to be herbivorous (e.g. rabbit, rat, guinea-pig). There is some variation in the anatomical relationships of the biliary and pancreatic systems, especially the site of entry of the pancreatic juice into the duodenum. These differences are illustrated in the table below.

species	gall-bladder	entry into duodenum		functional accessory panc. duct
		bile duct	main panc. duct	
man	yes	2nd part	2nd part	no
cat	yes	2nd part	2nd part	no
dog	yes	2nd part	2nd part	yes
rabbit	yes	2nd part	3rd part	no
rat	no	2nd part	2nd part	no
guinea-pig	yes	2nd part	3rd part	no

(Uddin and Case 1983)

(Mann 1919)

In the rat all pancreatic ducts drain into the common bile-pancreatic duct (BPD) which empties via a common channel into the duodenum (Richards 1964, Herriott 1964, Greene 1955, Lambert 1965, Kraus 1980). A few small ducts have been reported to drain individual pancreatic acini directly into the duodenum (Herriott 1965) but these are of little consequence in considerations of the whole pancreas. The pancreas itself consists of four segments - gastric, splenic, duodenal and parabiliary (Richards 1964, Pearson 1974). There are at least three major ducts and many minor ducts or ductules that join the bile duct at separate anatomical sites from the porta hepatis to the duodenum.

The weight of pancreatic tissue is directly related to the weight of the animal (Richards 1964). Richards (1964) noted that the most consistent pancreas weight, in terms of total body weight, was that of the total pancreas. It was for this reason that we measured total pancreatic gland weights in this study.

The pancreatic ducts, common bile-pancreatic duct and ampullary epithelium show considerable similarities when examined by histochemical and autoradiographic techniques (McMinn 1961). The ducts themselves comprise 4% of the weight of the gland, i.e. 1 g pancreas = 40 mg ducts (Bolender 1974). The large ducts and main BPD are lined with columnar epithelium with microvilli and occasional cilia. The smaller ducts progressively change their epithelium to cuboidal (Kodama 1983, Roberts 1972).

The microscopic appearance of rat pancreas is similar to that observed in man with multiple acini, islet cells, ducts and blood vessels. The

histological features of rat pancreas will be discussed at a later stage.

The greater part of the BPD is enveloped in pancreas but at each end there is an exposed segment 1-2 mm long, allowing free access to the duct. This structure of the biliary-pancreatic duct enables occlusion of bile flow near the liver to produce pure pancreatic flow down the BPD. Use of this is made in our experimental preparation for pancreatic infusion.

Anaesthesia

All animals were maintained at 37°C on a heating pad. A rectal thermometer checked temperature homeostasis.

Ether: (Diethyl-ether, B.P. May and Baker, Dagenham). was administered in a large sealable jar into which the rats were placed. Surgical anaesthesia usually occurred in less than one minute and was maintained by ether inhalation from a small jar throughout the operation. Ether administration whilst requiring constant vigilance and producing a variable depth of anaesthesia allowed a rapid post-operative recovery.

Sagatal: (Sodium pentobarbitone, B.P. May and Baker). 60 mg/ml was diluted to a 10% solution as recommended by Cruikshank (1965) and Berry (1981). A dose of 0.75mls/100 g body weight was administered intraperitoneally. Surgical anaesthesia occurred within 5 minutes and lasted for one hour. Further doses could be given by dripping sagatal directly onto

the liver during the operative procedure. The anaesthesia produced was of a constant depth and required little vigilance. Recovery was slow however, being up to 3 hours in some cases.

Inactin: (Sodium thiobutabarbitone, BYK, Germany).
This was administered to animals undergoing the non-recovery pancreatic duct mucosal barrier experiments described later. Given in a dose of 100 mg/kg intraperitoneally, anaesthesia occurred in 1-2 minutes. No metabolism of the drug is possible by the rat so the animals cannot recover from its administration. The anaesthesia produced was of a good level and no metabolic acidosis occurred within four hours. A routine tracheostomy was performed whilst using this anaesthesia to prevent airway obstruction.

Post-operative recovery

Following the operative procedure the animals made a full recovery. They were allowed free access to food and water with no restraint applied. The clinical state of the animals was assessed hourly following operation and any animals that died in the 24 hour period underwent immediate post-mortem examination. At 24 hours animals had developed moderate obstructive jaundice.

Sacrifice

At 24 hours the rats were reanaesthetized with ether and the laparotomy wound reopened. Peritoneal fluid was removed and stored for later amylase estimations. Any animal showing leakage from the duodenotomy was excluded from the results (in total 8 animals excluded). Macroscopic

assessment of the degree of pancreatic damage was based on fat necrosis, gland swelling, haemorrhage into the gland and the amount of peritoneal fluid. 2 mls of blood were taken from the inferior vena cava before manipulation of the pancreas and immediately centrifuged to separate the serum. This serum was frozen at -20°C for later biochemical testing. The animals were killed by bilateral pneumothorax and the whole pancreas removed. Two small representative portions were taken from the head and tail of the gland and placed in formalin for later histology. The gland was carefully weighed (wet weight) before being processed for either (i) water content estimation or (ii) homogenisation and estimation of enzyme content of the tissue (see later).

Operative procedure: The operation was performed under clean but not aseptic conditions. The abdomen was opened through an upper midline incision 2-3 cm in length. The bile-pancreatic duct (BPD) was carefully identified passing from the liver to the duodenum (fig. 3). At all times great care was taken to avoid pancreatic trauma during the experimental procedure. The BPD was ligated close to the liver (fig. 4A) and the animal left for 20 minutes to enable the BPD to clear of bile. Short term ligation of the bile duct and the subsequent production of jaundice has been previously shown not to interfere with the pancreas (Hansson 1967, Mosley 1981). In contrast Baba and colleagues (1983) have demonstrated that four weeks of obstructive jaundice induces enlargement of the pancreas due to hypertrophy and hyperplasia of acinar cells. Preliminary studies on ten rats confirmed that short term jaundice had no effect on the pancreas of our experimental preparation. The distal BPD was carefully dissected free (fig. 4B) and a loose ligature placed around it

immediately on entrance to the duodenum. A small duodenotomy was made opposite the entrance of the BPD and a thin polyethylene cannula (Portex 800/100/140, i.d. 0.4 mm, o.d. 0.8 mm) with a blunt metal tip was inserted through the ampulla of vater into the distal 2 mm of the BPD (fig. 4C). The cannula was loosely ligated in place. The cannula was attached to a three way connector (fig. 5) - one limb to a constant infusion pump (Constant infusion apparatus, Scientific and Research Instruments Ltd., Croydon, U.K.), and the other limb to a pressure transducer (see later for details). At the end of the experiment the cannula and ligature were removed, the duodenum closed with 7/0 suture (fig. 4D) and the abdomen in layers. Thus on recovery the animal had a ligature applied to the bile duct close to the liver only. The pancreas and duodenum were both intact. This preparation is similar to that described by Heinkel (1953), Donahue (1984) and Lankisch (1983) although it is important to note that these previous experiments did not use pressure measurements.

Volume

The volume used in these experiments was carefully measured in all cases by weighing (1 ml = 1 g). The results of these volume measurements at the various volumes are summarized below.

volume (µl)	measured volume (µl) (mean ± SD)
50	51±2
100	102±3
150	149±2
200	201±3
250	252±4
500	502±3
1000	1001±2

(N = 15 for each volume)

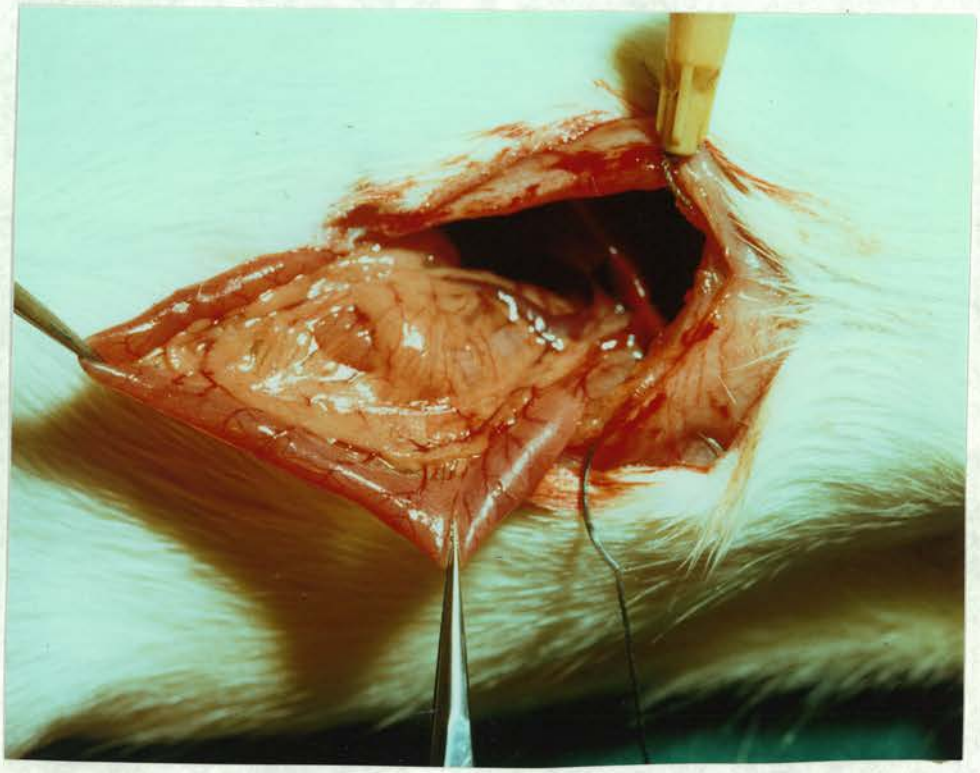


Fig. 3 The normal rat pancreas and surrounding structures.

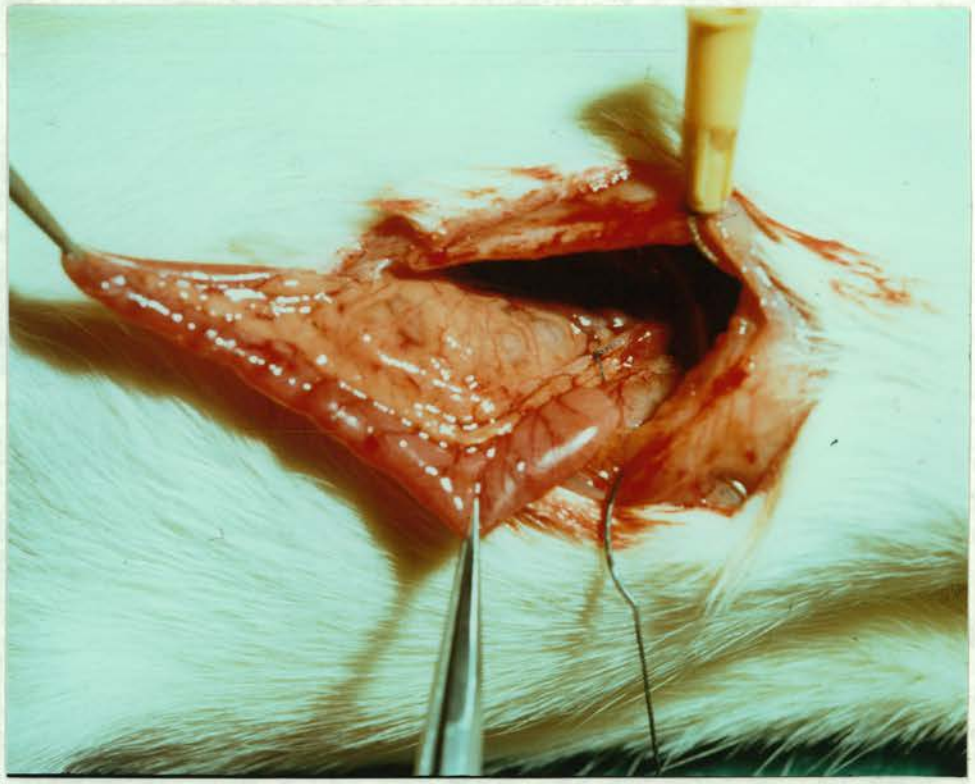


Fig. 4A Ligature on proximal Bile-pancreatic duct.

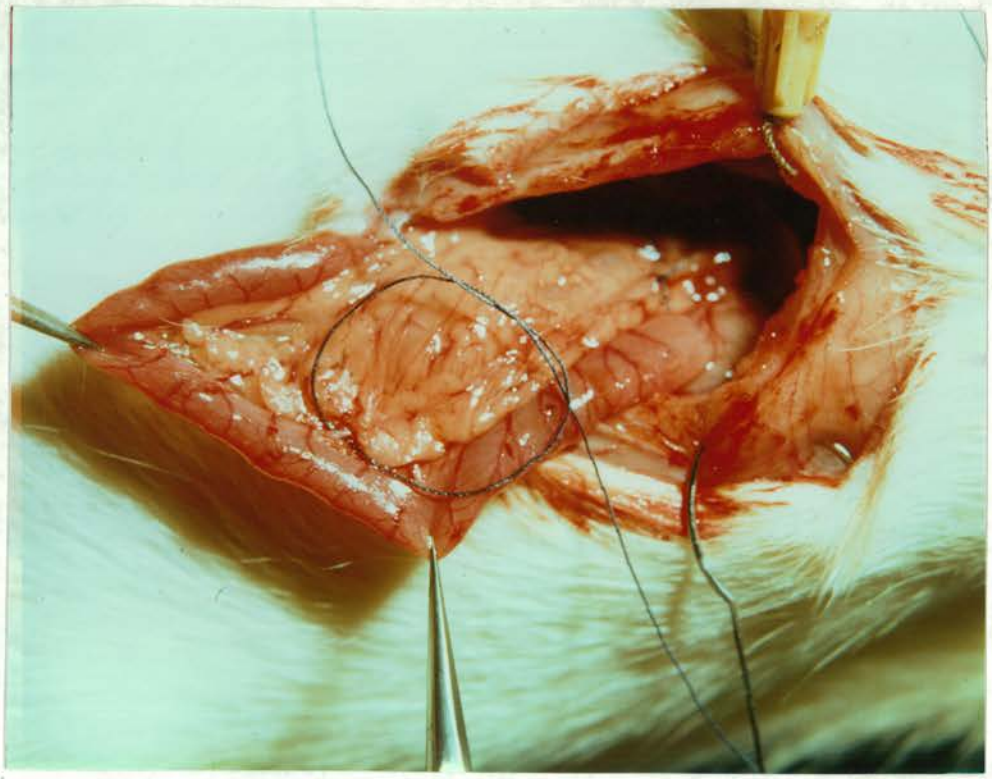


Fig. 4B Ligature around distal Bile-pancreatic duct.

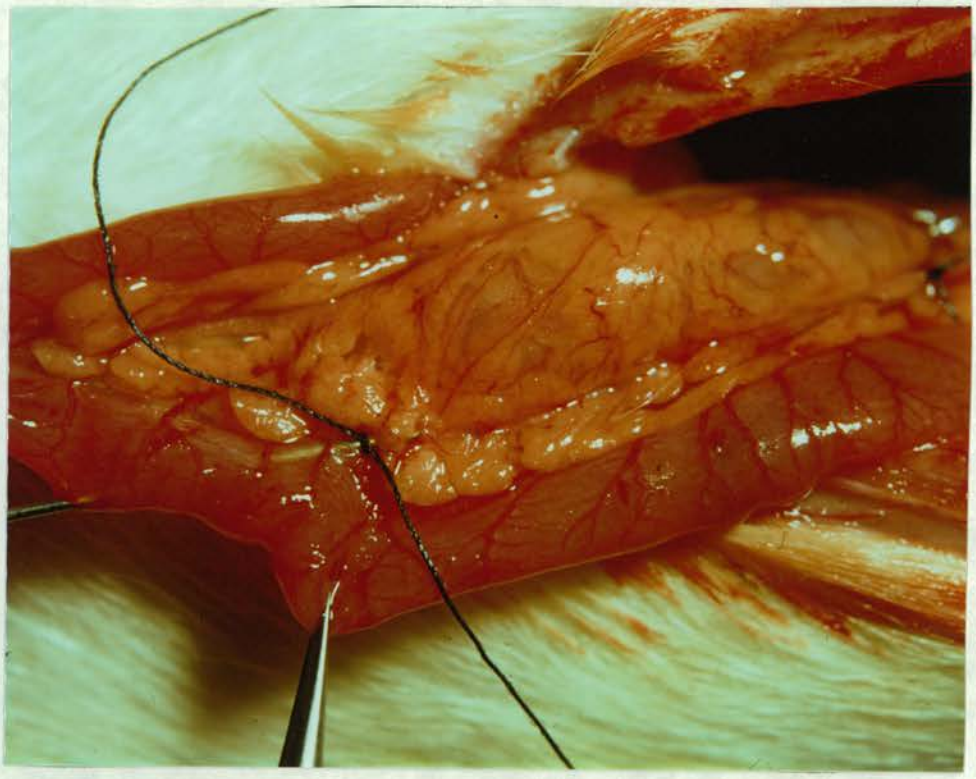


Fig. 4C Cannula in Bile-pancreatic duct through transduodenal approach.

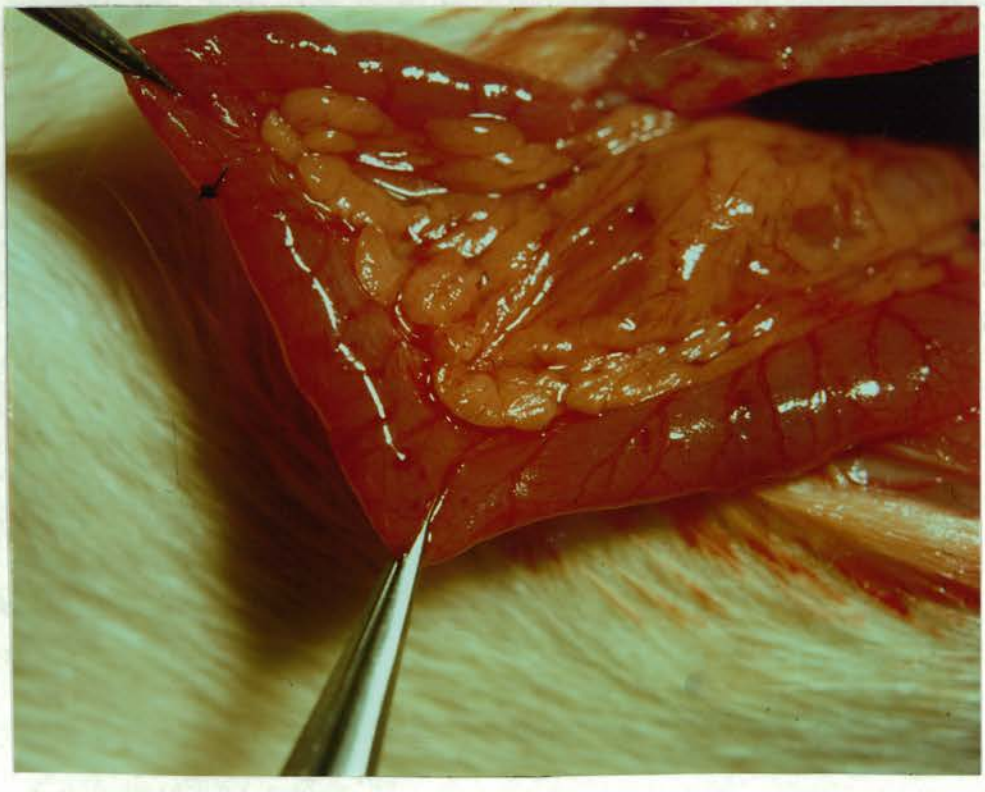


Fig. 4D Cannula removed. Duodenotomy closed.

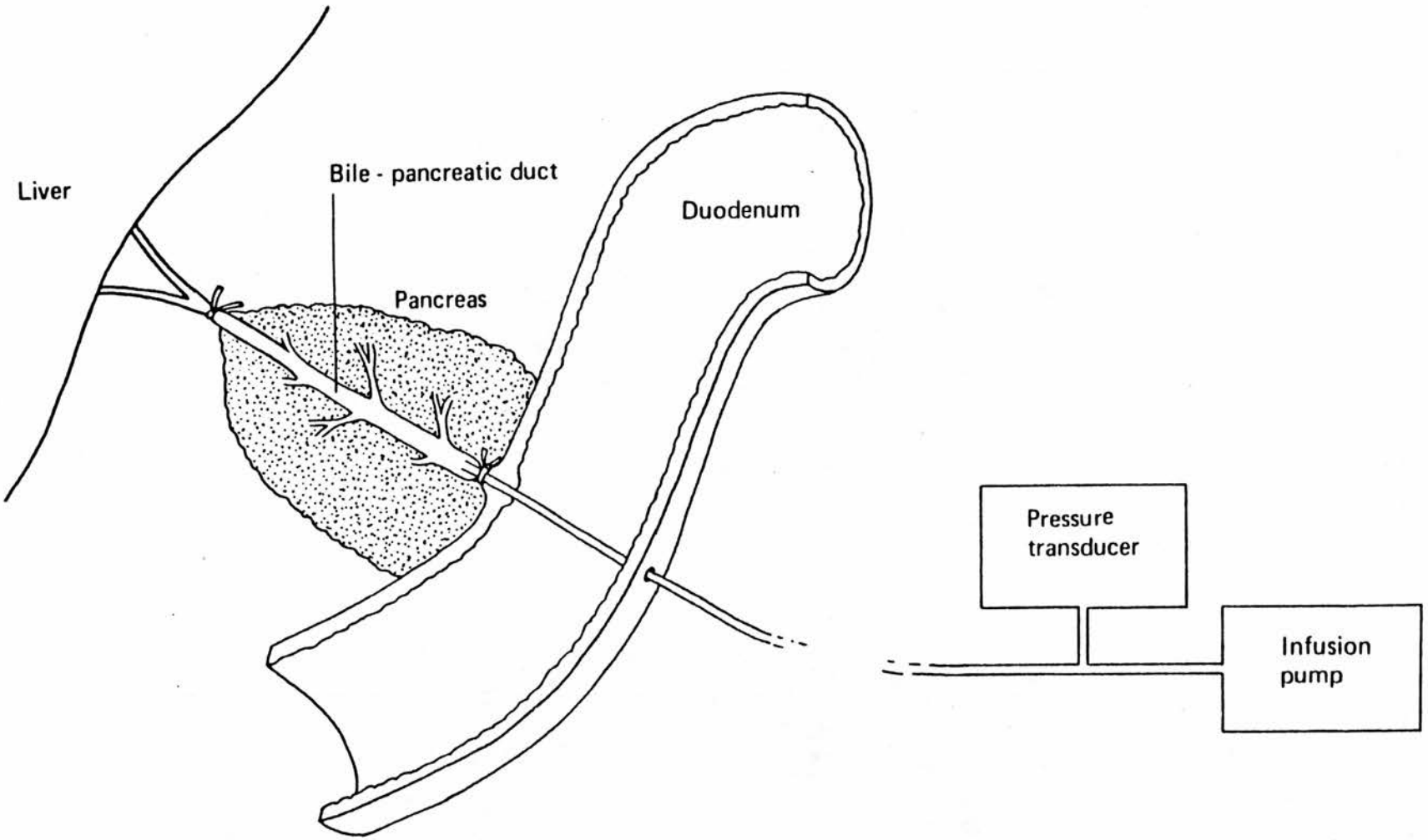


Fig. 5 Experimental preparation.

Pressure

Pressure measurements (in cm H_2O) were performed using a Statham transducer connected to a mingograf recorder (Elema-Schönander, Sweden). The results obtained were checked by using a Bell and Howell transducer (type 4/422) (Basingstoke, England) connected to a Lectromed recorder (Jersey, Channel Islands). The pressure recordings between the two systems demonstrated 100±5% concordance.

Control animals

Two groups of animals were used for comparative purposes.

(i) sham: animals undergoing laparotomy and manipulation of the pancreas only without cannulation of the ducts.

(ii) true control: animals undergoing cannulation of the duct but without infusion of fluid.

Bilirubin measurements

These were performed in all animals to ensure that the bile duct was properly occluded and no endogenous biliary contamination was possible. (Mean value ± SD in normal rats = 8.4 μmol/l ± 2.9). The mean value at 24 hours in rats undergoing experimentation was 64 ± 8.6 μmol/l. In animals where the bilirubin did not rise by threefold or more the results were discarded (six animals in all).

Pancreatic damage

The pancreatic damage at 24 hours was assessed in several ways.

(i) pancreatic gland weights.

(ii) water content of gland.

(iii) amylase levels - serum

- peritoneal fluid

- gland tissue.

(iv) histology.

(i) pancreatic gland weights (PGWR).

To obviate the ~~discrepancies~~ variations in body weight the wet weight of the pancreas was combined with body weight to produce the pancreatic gland weight ratio (PGWR) (mg/100 g). Previous figures for Sprague-Dawley rats quoted in the literature are:

350±20 (mg/100 g) (Richards 1964).

337±8 (mg/100 g) (Baba 1983a).

297±25 (mg/100 g) (Baba 1983b).

(ii) water content of the gland (WC). This was a measurement of chemical oedema in the pancreas. This was measured in animals undergoing pressure studies only by the method of Aho (1983). The wet weight of the pancreas is carefully measured as previously. For the measurement of dry weight each pancreas was fixed in ethanol for 10 hours and then dried to a constant weight at 60°C. The oedema was expressed as the water content of pancreatic tissue.

i.e. water content (%) = $\frac{\text{wet wt. - dry wt.}}{\text{wet wt.}} \times 100$

The value for normal pancreatic tissue obtained in this study was 68±1.7% (mean ± SD). Aho (1983) quotes a similar value of 68.5% for normal glands.

(iii) serum amylase (SA).

Blood taken from the IVC was immediately centrifuged and

the serum stored at -20°C. Amylase estimation was performed by the Phadebas (Pharmacia, Uppsala, Sweden) method. The value obtained for normal animals was 1120±468 U/l, - a value close to that reported by other authors (Lankisch 1979a, Huttenen 1973). It should be noted that the basal serum amylase level in the rat is five to six times greater than that seen in man.

peritoneal fluid amylase (PFA).

The peritoneal fluid was removed from the peritoneal cavity and stored at -20°C. Amylase estimations were performed using the Phadebas technique (N.B. high dilution factors used because of the extremely high levels). In animals undergoing sham laparotomy only no fluid could be detected.

gland amylase (GA).

In an attempt to define the severity of pancreatic damage the pancreatic tissue amylase level was measured. The pancreas was removed and dissected as free of fat as possible (Boctor, 1981). The gland was homogenised in 6 mls of pre-cooled (+4°C) 0.01 N HCl containing 0.01 mol/l $CaCl_2$ (Huttenen, 1973). Homogenisation was performed with a hand electric drill using a glass tissue homogenizer fitted with a teflon pestle. The homogenate was centrifuged at 160,000 g for 1 hour at 4°C. The clear supernatant was removed and kept at -20°C until use. The amylase content of the homogenate was determined by the Phadebas method (u/l).

The protein content was assayed according to the method of Lowry et al (1951) using bovine serum albumen as the reference protein (mg/l). The amylase was expressed as u/mg of protein. The value obtained for a normal pancreas was 12.6±6.8 u/mg, similar to that quoted by Huttenen (1973). For reasons explained later this estimation was not performed for all animals.

(iv) Histology

Representative portions were taken from the head and tail of the gland. The tissue was fixed in formalin, dehydrated in ascending strengths of ethanol, cleared in cedarwood oil and embedded in paraffin. Some specimens, used for later photography, were embedded in plastic resin. Sections (5 μ thick paraffin, 3 μ plastic) were cut with a microtome and stained with haematoxylin and eosin. Each section was coded and assessed by two independent observers. The correlation between the two observers was good ($r = 0.95$, $P < 0.001$).

Several methods of histological assessment of pancreatic damage have been described. Hansson (1967) assessed several histological features and graded them on a scale 0 to +++ i.e. acinar necrosis, bleeding, thrombosis, interstitial inflammation, dilatation of acini and the amount of zymogen granules in the acinar cells, peripancreatic tissue, fat necrosis and inflammatory reaction. The acinar necrosis was classified as a small solitary necrosis of parenchymal cells, ++ several foci of necrotic parenchyme, +++ widespread

areas of necrosis. Nevalainen (1975) evaluated various microscopic changes in the rat pancreas following formation of a closed loop experimental model of acute pancreatitis. i.e. oedema, haemorrhage, peripancreatic fat necrosis, inflammatory cell infiltration, acinar cell necrosis, ductal dilatation and loss of basophilia. Rao (1981) classified the degree of pancreatitis as mild, moderate to severe and severe haemorrhagic based on changes related to oedema, inflammatory cell infiltration, vascular change, ductal changes and acinar changes. Thus quantification of the severity of acute pancreatitis was possible. Several other authors have attempted to grade pancreatic changes on a scale 1 to 3 and thus develop a pathological scoring system (Pissiotis 1972, McCutcheon 1963, Anderson 1965, Pfeffer 1962, Reinitz 1977, Jalovaara 1978, Poncelet 1972).

In this study we have used a pathological scoring system based on the observations of Hansson (1967), Nevalainen (1975) and Rao (1981). Five histological features were assessed:

- acinar necrosis
- oedema
- duct changes
- inflammatory infiltrate
- haemorrhage

Each of these features was scored 0-3:

0 = normal

1 = mild changes

2 = moderate changes

3 = severe changes

These features were assessed both in the head and tail portions of the gland and a mean value obtained. Thus each gland had a final score of 0-15 with a score of

- 0-5 representing mild pancreatic damage,
- 6-10 representing moderate pancreatic damage, and
- 11-15 representing severe pancreatic damage, or acute haemorrhagic pancreatitis (AHP).

This classification corresponds closely to that used in the human setting, where Kummerle and Hollender (1983) have graded acute pancreatitis into

I - oedematous or mild

II - partly necrotizing or moderate

III - AHP or severe

Examples of pancreatic damage are shown later in this thesis.

Correlation of parameters of pancreatic damage.

The histological damage observed in the pancreas was taken as the "gold standard" against which all the other parameters were assessed. Correlations between the parameters are given below using Pearson's "r" value.

	r value	p value
PGWR v water content	0.82	$p<0.001$
PGWR v histology	0.75	$p<0.001$
PGWR v histological oedema	0.40	$p<0.001$
water content v hist. oedema	0.91	$p<0.001$
SA v histology	0.34	$p<0.02$

	r value	p value
PFA v histology	0.64	$p < 0.001$
gland amylase v histology	0.17	$p > 0.10$

There thus appeared to be no relationship between the pancreatic gland amylase level and the histological changes observed. In view of this finding we abandoned use of this time consuming investigation. The parameters of pancreatic damage used in this study were:

(i) pancreatic gland weight ratio (PGWR)
(also water content in pressure studies).

(ii) serum amylase. (SA)

(iii) peritoneal fluid amylase. (PFA)

(iv) histology score.

Statistical Analysis

For analysis between groups of animals (N usually 8 or 10) the Mann-Whitney U test or Student's t test was used. Correlation using Pearson's "r" was used to compare the relationship between two variables. Statistical significance only occurred when $P < 0.05$ (i.e. 5% level).

CHAPTER III

INTRADUCT VOLUME AND THE PANCREAS

Introduction

One of the pre-requisites for the development of an experimental preparation which functions within physiological limits in the study of acute gallstone pancreatitis is that the volume of infusate be carefully controlled. The other major pre-requisite, that of pressure of infusion, is discussed at length in the next chapter. Most experiments using the rat have infused various solutions into the pancreatic ductal system to produce pancreatitis. Whilst this method of producing pancreatic damage has much to commend it there has been little quantification of the volume of infusate. Indeed pancreatitis can easily be produced by infusing a large volume of various substances (e.g. olive oil) at high pressure into the pancreatic duct. However this situation is irrelevant in the clinical setting as only a small volume is likely to reflux into the pancreatic duct, and that at a fairly low pressure. A review of the volumes of infusate into the pancreatic duct system of the rat used by previous authors is summarised in Table 1. The diversity of volumes used ranges from 100 µl (Terry 1982, Ohnishi 1984) through 200 µl (Donahue 1984, Aho 1982) and 500 µl (Evander 1981) to the large volumes of 1000 µl (Poncelet 1972) and 1500 µl (Hansson 1967). Comparison between the degrees of pancreatic damage induced by such infusions is impossible.

In the human setting measurement of the volume of the pancreatic ducts has become possible with the development of the radiological technique of ERCP (Endoscopic Retrograde Cholangiopancreatography). Filling of

TABLE 1 The variety of volumes infused into the rat pancreas

Volume	Fluid Infused	Reference
100µl	Enterokinase/TC	Terry, 1982,1983
	trypsin/TC	Ohnishi, 1984
200µl	FROP's/bile salts	Anderson, 1983
	TC	Aho, 1980a,b,1982
	TC	Demol, 1983
	trypsin	Papp, 1973
200-250µl	TC	Aho, 1983
200-500µl	DOC	Olazabal, 1980
300µl	'alcoholic' bile	Gamklou, 1966
400µl	'alcoholic' bile	Jalovaara, 1978
500µl	DOC	Huttenen, 1973
	DOC	Heinkel, 1953
	TC/T.DOC	Greuter, 1981
	T.DOC/trypsin	Arnesjö, 1971
600µl	TC	Lankisch, 1974,1979a,b, 1983
		Gabryelewicz, 1983
1000µl	lysolecithin, etc	Poncelet, 1972
1500µl	Enterokinase	Mann, 1979
1500µl	bile salts	Hansson, 1961,1967

TC - Taurocholate

DOC - Deoxycholate

T.DOC - Taurodeoxycholate

FROP's - Free radical oxidation products

the entire ductal system is usually achieved with a volume of 2-5 mls (Kasugai 1974, Stewart 1977, Waldron 1968, Hermann 1979, Anacker 1977). If a larger volume is infused there is either escape into the duodenum or a pancreatogram with filling of the small ducts results. It is not infrequent when a large volume of contrast is used during ERCP for duct rupture to become apparent with the accompanying sequelae of acute pancreatitis. Elmslie and coworkers (1963, 1966) have shown that injection of 0.75 ml of 50% Hypaque (sodium diatrizoate, BP) ensures complete filling of the pancreatic duct in 10-15 kg dogs, and that larger volumes caused extravasation of contrast into the gland. Thus the maximum volume of a solution that can be accommodated in the adult dog pancreatic ducts without causing rupture of the ductules is 0.75 ml.

The normal human pancreas weighs 100-150 g (Hermann 1979, Geokas 1983) and the rat pancreas weighs approximately 1 g (Richards 1964, Herriot 1963), giving a 100:1 ratio between the pancreases of man and rat. Using this ratio to calculate the equivalent volumes of pancreatic ducts approximates to 5 mls (man): 50 µl (rats) (Table 2). As 5 mls is the maximum volume of the pancreatic duct then the physiological volume of infusate into the rat pancreas must be less than 100 µl. It was these observations that stimulated a study of the effect of infusate volume on the pancreas.

Materials and methods

Object

The object of this initial study was to establish the volume of injectate which would be physiological for the rat preparation used.

Volume injected into Rat (Pancreas 1g)	Human equivalent (Pancreas 100g)
μl	ml
50	5
100	10
150	15
200	20
500	50
1000	100

TABLE 2 Comparative Volumes

Experimental preparation

The experimental preparation previously described in figure 5 was used. Sterile Indian ink was passed through a 4 micron filter and diluted to a final osmolality of 300 mosm/kg (the same as that of rat plasma).

Infusion - volume study

Indian ink solution was infused into the rat pancreas at a rate of 50 µl/minute. Throughout the infusion the pressure within the system was measured and the maximum pressure reached with each volume recorded. Varying volumes were infused into the pancreas : 50 µl, 100 µl, 150 µl, 200 µl, 500 µl, 1000 µl. (N = 5 for each).

Pressure study

The results obtained from the volume studies suggested further study using different pressures of infusion with a constant volume. 50 µl Indian ink was infused into the gland at maximum infusion pressures of 10, 15, 20, 25 and 50 cm H_2O by varying the rate of infusion (N=5 for each group).

Assessment

At 20 minutes the animals were sacrificed and the whole pancreas removed. The gland was assessed macroscopically for the degree of acinar blackness by two independent observers using a scale 0-3 (for both head and tail of gland).

0 = no blackness.

1 = mild blackness.

2 = moderate blackness.

3 = severe blackness.

Two representative portions were taken from the head and tail of the pancreas. These were processed as previously described and haematoxylin and eosin sections prepared. The histological appearance was assessed for ductal extravasation of Indian ink by the same two observers and graded 0-4.

0 = Indian ink - all in ducts.

1 = Indian ink - little outwith ducts but no ruptures.

2 = Indian ink - few duct ruptures.

3 = Indian ink - moderate extravasation with few duct ruptures.

4 = Indian ink - severe extravasation with multiple duct ruptures.

Results

The results of infusing different volumes of Indian ink are shown in table 3.

50 µl volume: the highest pressure reached in the system was 15 cm H_2O. Macroscopically Indian ink remained in the ducts and there was no evidence of acinar staining (fig. 6A). Histological changes of duct extravasation were minimal (see later) and were more marked in the head.

100 µl volume: the highest pressure attained was 43 cm H_2O. There was macroscopic evidence of acinar staining throughout the gland (fig. 6B). Histological ink extravasation was marked with much of the Indian ink lying free outwith the ducts from areas of duct rupture.

150 µl volume: the highest pressure recorded was 62 cm H_2O. There was however a sudden fall at this pressure to a value of 35 cm H_2O, indicating sudden ductal disruption. Marked acinar staining was noted throughout the gland. Histological extravasation was prominent with ductal rupture commonly seen.

Volumes 200-1000 µl: these volumes were associated with similar appearances

Volume (μl)	Highest Pressure (cm H_2O)	Macroscopy (0-3)		Histology (0-4)	
		head	tail	head	tail
50	15	0	0	1.1	0.2
100	43	2.1	1.2	2.3	1.7
150	62	2.9	2.4	3.1	2.8
200	81	3.0	3.0	4.0	4.0
500	90	3.0	3.0	4.0	4.0
1000	93	3.0	3.0	4.0	4.0

TABLE 3 Indian Ink infusion : volume, pressure and changes produced

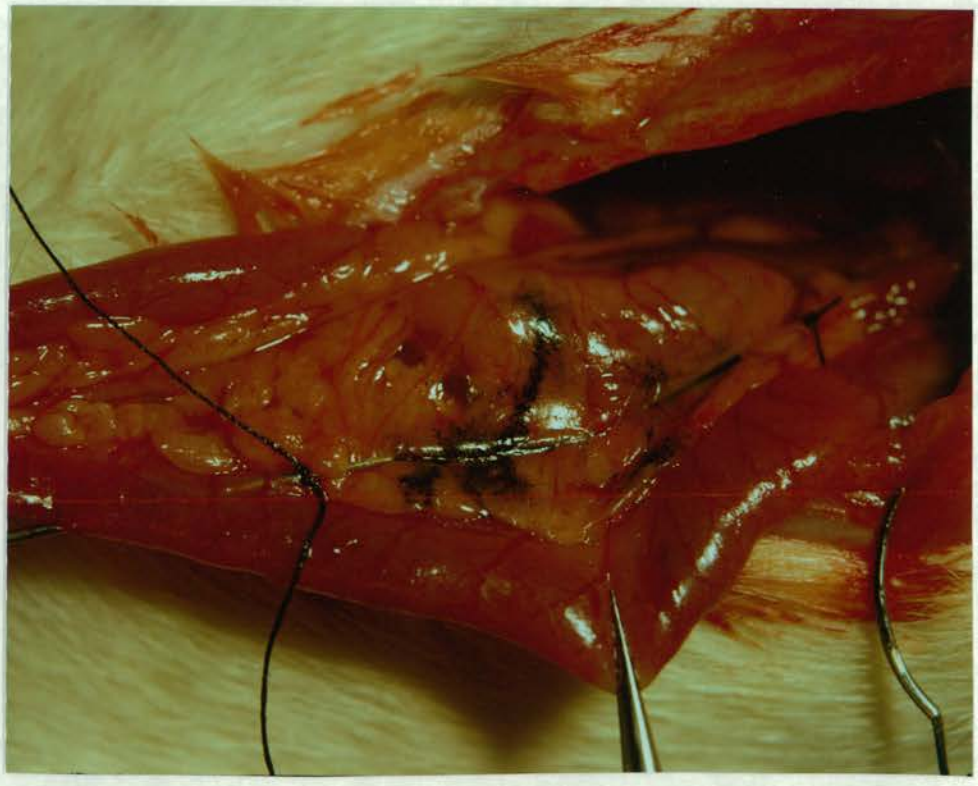


Fig. 6A Infusion of 50 µl Indian ink.

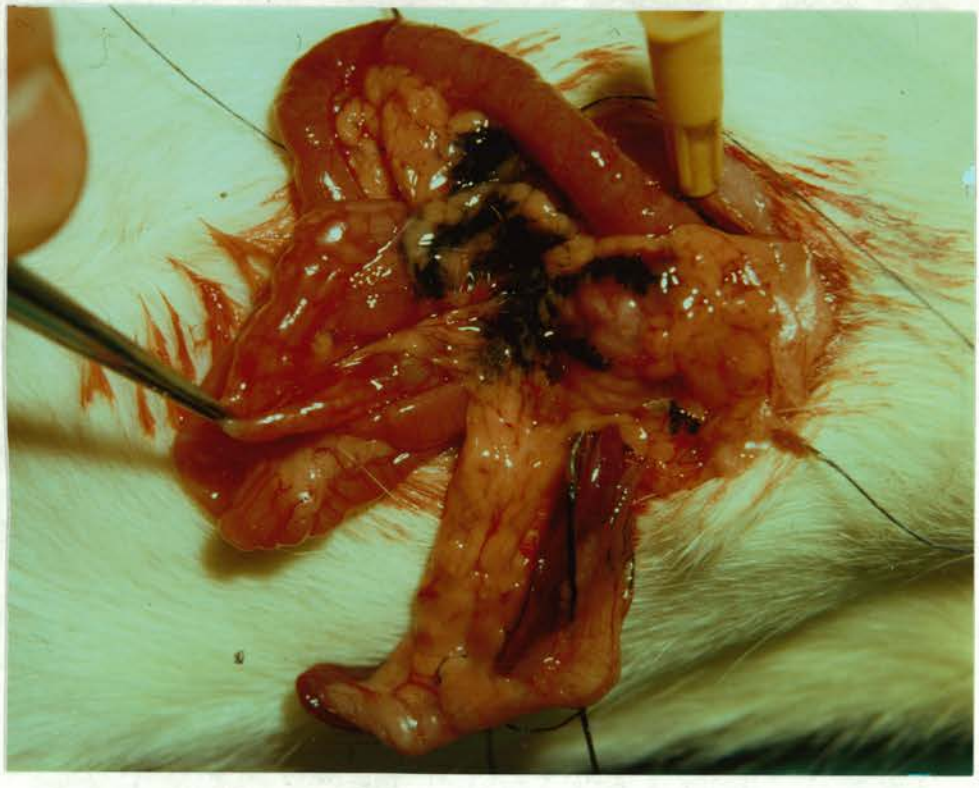


Fig. 6B Infusion of 100 µl Indian ink.

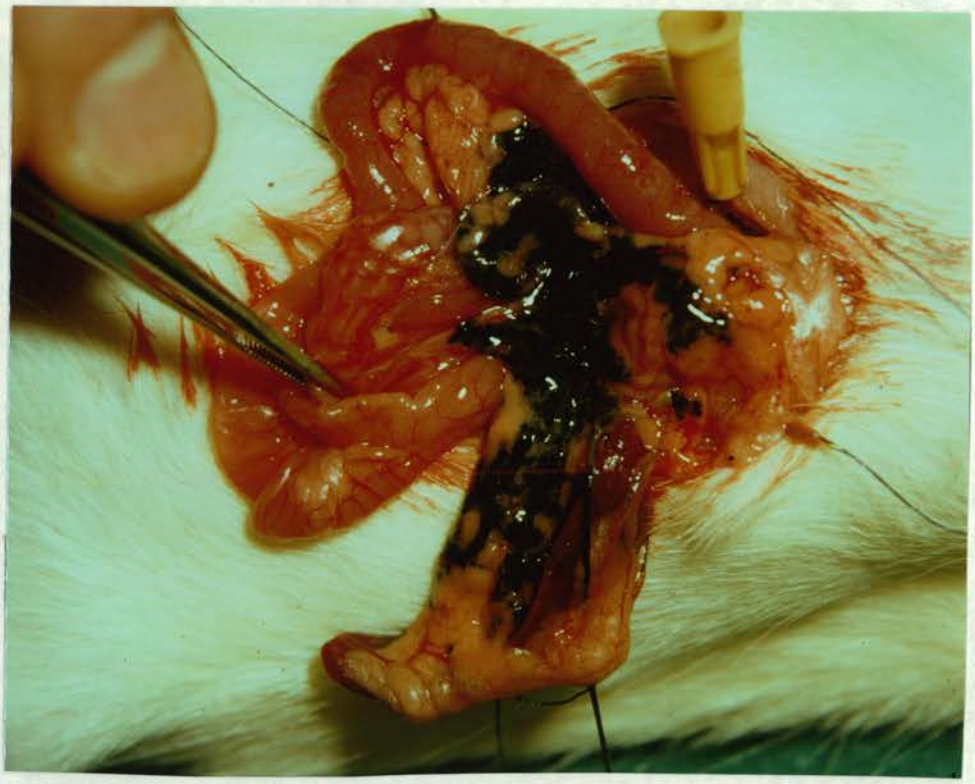


Fig. 6C Infusion of 200 µl Indian ink.

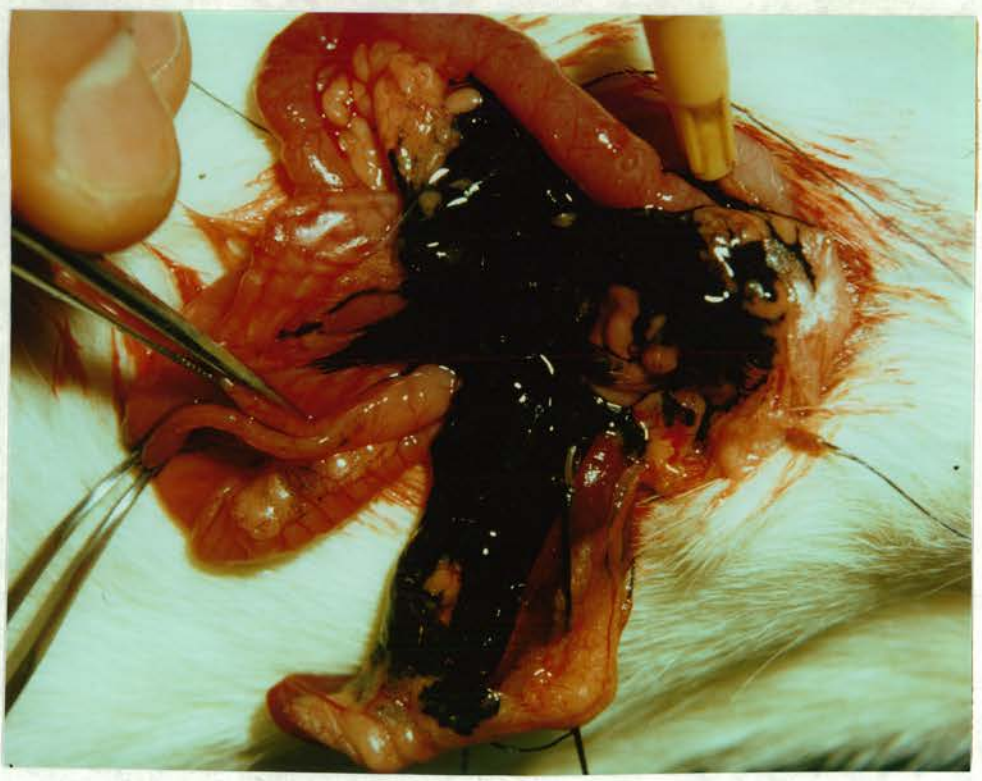


Fig. 6D Infusion of 1000 µl Indian ink.

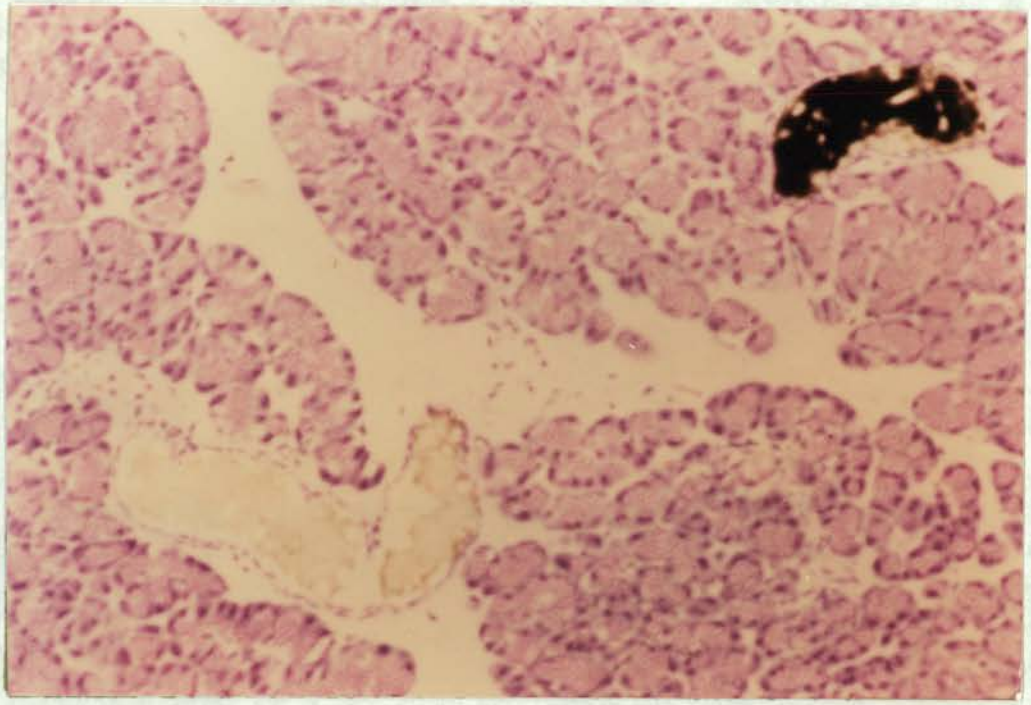


Fig. 7A Histology: Indian ink all in ducts (H+E x 200).

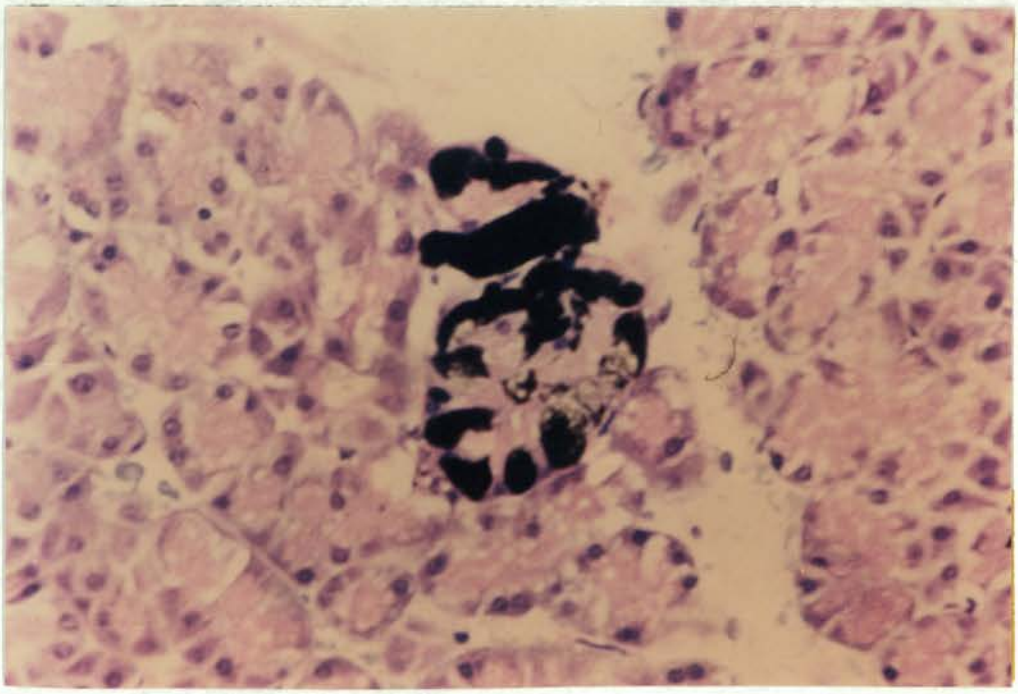


Fig. 7B Histology: Indian ink passing through intercellular clefts (H+E x 250).

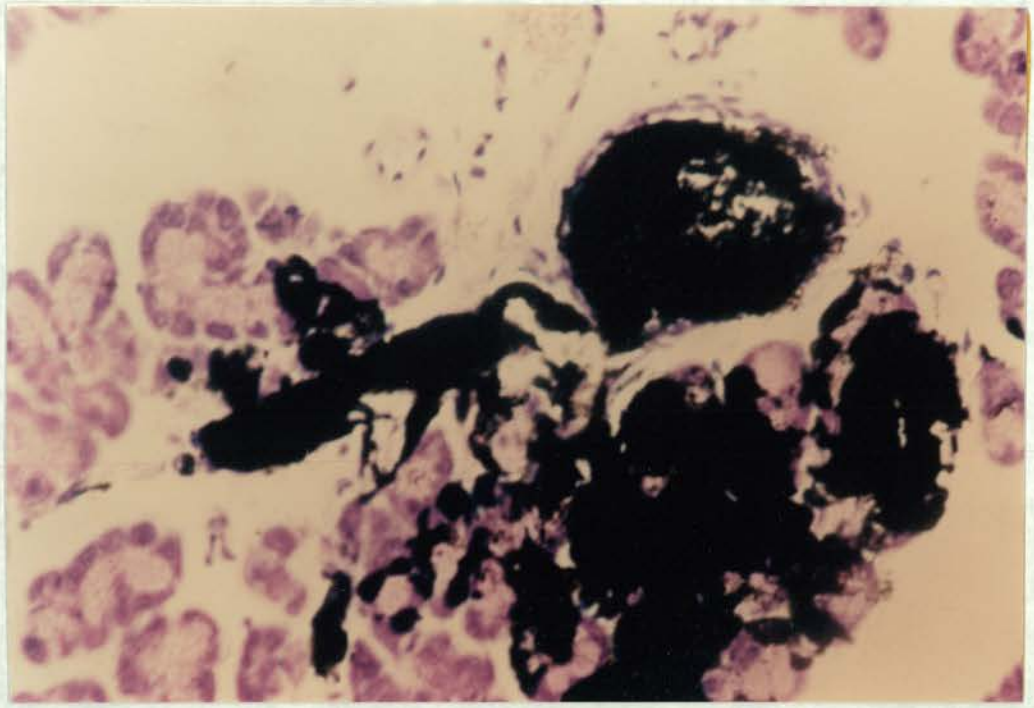


Fig. 7C Histology: Indian ink passing through large duct rupture (H+E x 250).

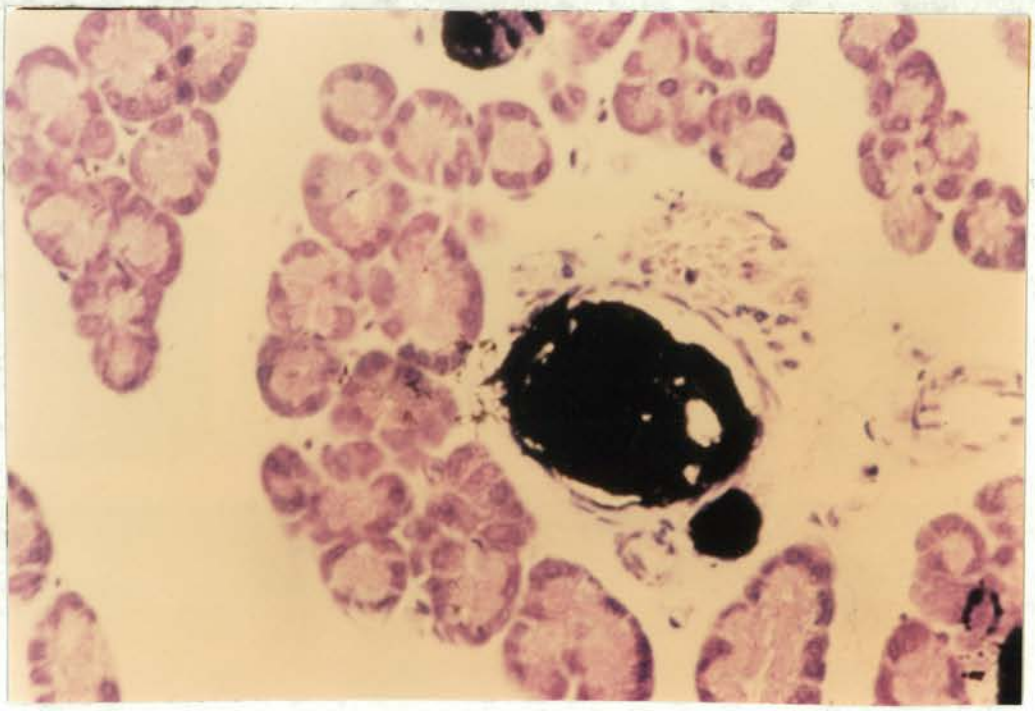


Fig. 7D Histology: Indian ink passing through small duct rupture (H+E x 250).

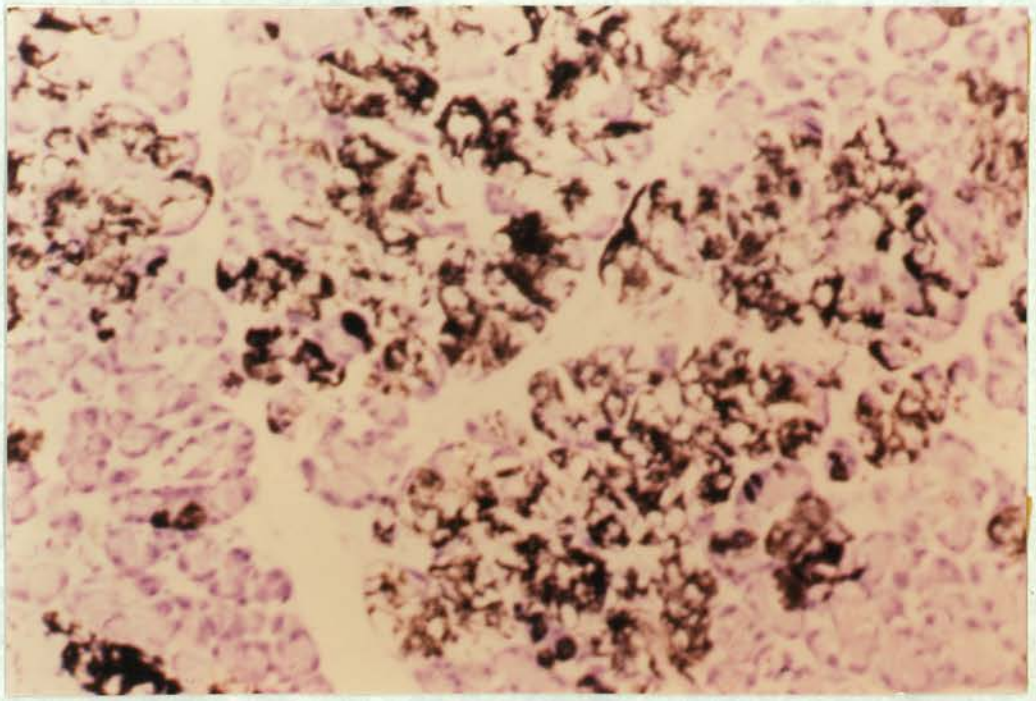


Fig. 7E Widespread extravasation of Indian ink (H+E x 200).

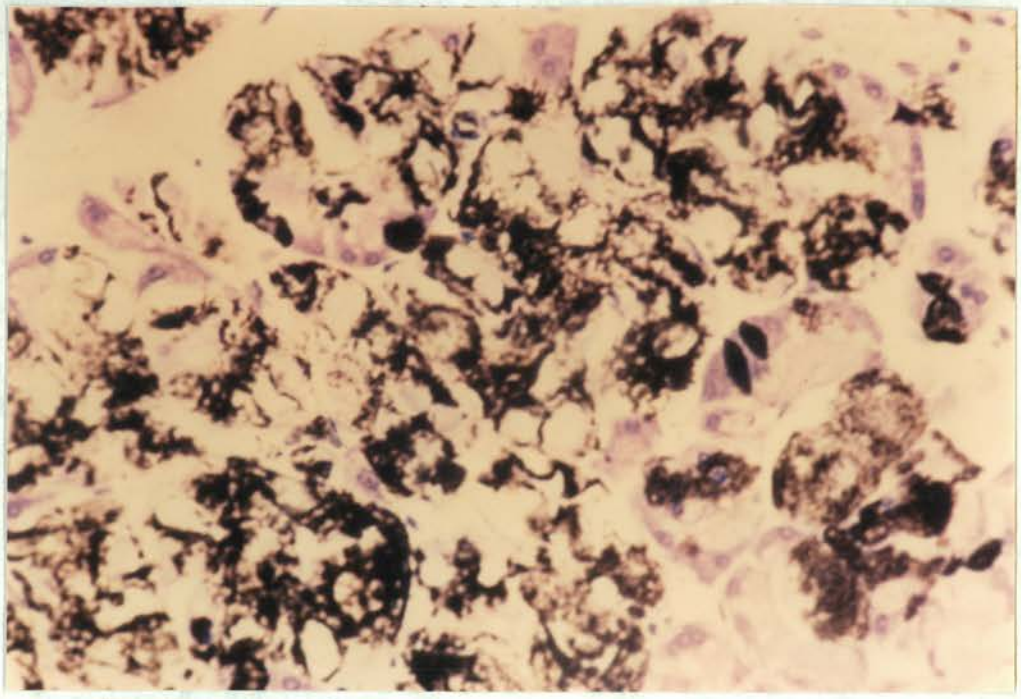


Fig. 7F Widespread extravasation of Indian ink (H+E x 250).

in the pancreas. The maximum pressures reached were 200 µl -81 cm H_2O, 500 µl - 90 cm H_2O and 1000 µl - 93 cm H_2O. These pressures were, however, only reached transiently as there was a sudden fall to values of 30-40 cm H_2O indicating gross ductal disruption. Macroscopic heavy staining of the entire gland was noted in each case (figs. 6C and 6D). The histological appearances were those of gross ductal disruption with Indian ink free throughout the gland.

Pressure studies: (50 µl volume)

10, 15 cm H_2O: all the Indian ink was within the ductal system (fig. 7A). There was no evidence of acinar staining or ductal extravasation.

20, 25 cm H_2O: a mild to moderate degree of acinar staining was noted in the gland tissue. Histologically Indian ink appeared to be leaving the ducts via intercellular clefts into a periductular and periacinar space (fig. 7B).

50 cm H_2O: a moderate degree of acinar staining was observed. Histological evidence of duct rupture was apparent with Indian ink free in the periductal space (figs. 7C and 7D).

Figures 7E and 7F demonstrate extravasation of Indian ink using the 200 µl volume.

Discussion

This initial research is important in the study of acute gallstone pancreatitis as the volume of fluid refluxing into the pancreatic duct of man is limited by the volume of the duct and the pressure of reflux. In the experimental situation the injection of a large unphysiological

volume, at grossly elevated pressures, has limited relevance in a meaningful study of acute gallstone pancreatitis. Indian ink was used as a marker of ductal integrity with the use of this compound being well established in the study of ductal structure (Pirola 1970, Bockman 1971, Edlund 1963, Egdahl 1968, Anderson 1968). Other compounds such as fluorescein (Duprez 1963), ferritin particles (Bockman 1971) and various dyes (Mallet-Guy 1958) have been used in similar studies. This study has shown that injection of a volume of 100 µl or more into the pancreas of a rat is associated with duct rupture and gross extravasation of the duct contents. 100 µl in the rat is equivalent to 10 mls in the human, well in excess of human pancreatic ductal volume. These results are in contrast to those obtained when the 50 µl volume was studied. Using this physiological volume there was a close relationship between ductal extravasation and infusion pressure. At low pressures (10, 15 cm H_2O) no ductal extravasation was seen. At moderate pressures (20, 25 cm H_2O) extravasation was identified between the acinar cells but no evidence of duct rupture was seen. At high pressure (50 cm H_2O) there was evidence of duct rupture. These results are similar to those of Pirola (1970) although he did not quantitate the volume of infused Indian ink carefully. It is important to note that Indian ink can escape from the ducts through intercellular clefts at physiological pressures, confirming the earlier observations of Pirola (1970) and Egdahl (1958). At high pressure (>35 H_2O) this study confirms that ducts rupture with escape of pancreatic secretions (Pirola 1970, Dreiling 1952, White 1966).

This study has therefore demonstrated that 50 µl is the optimum volume for infusion into the rat pancreas. As the volumes of infusate used in previous studies have all been of 100 µl or above, interpretation and

extrapolation of such results to a meaningful study of acute gallstone pancreatitis remains questionable. On the basis of this initial research a volume of 50 µl was used in all further infusion studies.

Conclusions

1. The volume of infusate is important in producing ductal extravasation.
2. All volumes of 100 µl or above produce duct rupture and are considered unphysiological when the rat is used as an experimental model.
3. Duct extravasation produced by the 50 µl volume is dependent on infusion pressure. At low pressures there is no extravasation. At moderate pressures Indian ink passes through intercellular clefts and at high pressures there is evidence of duct rupture.
4. 50 µl volume should be used in infusion experiments with the rat pancreas.

CHAPTER IV

PRESSURE AND THE PANCREAS

Introduction

Although the pathogenesis of acute gallstone pancreatitis (AGP) remains an enigma there appear to be several contributing factors. The finding of gallstones in the faeces of more than 80% of patients following an attack of acute gallstone pancreatitis (Acosta 1974, Kelly 1976) suggests that there is passage of gallstone down the biliary tree to the ampulla of Vater. On reaching the ampulla temporary occlusion may occur leading to a rise in pressure in the bile and pancreatic duct system (fig. 1). The amount of reflux between these two ducts is dependent on intrinsic pressure relationships. The gallstone may then pass into the duodenum with associated temporary incompetence of the ampulla and consequent duodeno-pancreatic reflux (fig. 2). Proponents of these two theories have been numerous. The bile reflux theory of Opie (1903) has been hotly disputed by those suggesting duodenal reflux to be more important (McCutcheon 1968, Johnson 1967). The third theory of obstruction of the pancreatic duct in the presence of an actively secreting gland (obstruction-hypersecretion theory) has fallen into disfavour (Eckhauser 1981).

It is important to emphasize that a common feature of these three theories is the pressure relationships within the pancreatic - biliary - duodenal system. If pancreatic reflux were to occur from either the biliary system or duodenum then the amount of reflux would be dependent on pressure differentials. Moreover, it has been postulated (Klein 1983, Banks 1971) that an increase in pressure within the pancreatic duct

might be an aetiological mechanism in the genesis of AGP, an increase in pressure leading to rupture of ducts (Pirola 1970) and escape of ductal contents into the interstium (Edlund 1963).

This study explores the pressure relationships between the pancreatic and bile ducts and duodenum.

Factors that determine pressures in biliary and pancreatic ducts. (Hallenbeck 1967).

Pressures in the biliary and pancreatic ducts are directly proportional to the pressure and the rate at which fluid enters the system and inversely proportional to the resistance of flow through it. Pressures in these ducts, as with pressure in other abdominal viscera, are modified by intra-abdominal pressure, which varies in turn with respiratory movements. The greatest possible pressure available to propel bile or pancreatic juice through the ducts are the maximal secretory pressures of the respective organs. Resistance to flow is influenced by many factors, including the length and calibre of the ducts and the viscosity of the fluid, but the principal regulators of resistance in the biliary and pancreatic ductal systems are the sphincteric structures that surround the terminal portions of the ducts.

Secretory pressures of liver and pancreas

Cannulation of a bile or pancreatic duct allows measurement of the maximal secretory pressures of these two organs. The pressures attained exceed pressures that could result from simple diffusion from capillary blood; an active secretory process is involved (Hallenbeck 1967, Brauer 1954, Davenport 1976). The secretory pressures of the liver

and pancreas do not indicate the absolute force that the secretory cells of these organs can generate but rather the pressure at which the maximal rate of secretion is balanced by retrograde escape of the secretion through the organ and into its lymphatics. Thus the secretory pressure of the liver or pancreas can be regarded as a measure of leakiness of the organ, and it is of interest both that pancreatic secretory pressure in a dog with fibrosis and atrophy of the pancreas induced by chronic obstruction was far above normal (Wulsin 1953), and that hepatic secretory pressure of rats with livers made cirrhotic by exposure to carbon tetrachloride were significantly higher than normal (Shorter 1959).

When ductal obstruction is maintained, biliary secretory pressures have been observed at or near maximal levels for at least 6 days in the cat (Mitchel 1916) and for at least 4 days in the rat (Shorter 1959). Pancreatic secretory pressure in rats remained maximal after 24 hours of obstruction (Grossman 1958). There is now evidence that after occlusion of the common channel, bile duct pressure may increase above that in the pancreatic duct (Herriott 1966, Hansson 1967); an observation of relevance to the passage of gallstones down the biliary tree.

Secretory pressures have been measured during obstruction of the bile and pancreatic ducts. When the hepatic secretory pressures are measured the gall bladder, if present, must be removed, or the cystic duct ligated, in order to obtain values uninfluenced by the elasticity and absorptive capacity of the gall bladder. Values obtained when the animals are anaesthetized tend to be lower than when the same

animal is unanaesthetized (Hallenbeck 1967, Sewell 1976). A summary of these values is given below.

(mean values) (cm H_2O)

	liver	pancreas	reference
rat	21.5		Mann 1918
	24.0		Shorter 1959
		22.1	Grossman 1958
		.....	
dog	30.3	31.3	Herring 1907
	17.6	18.3	Gilsdorf 1967
	32.5	22.1	Hallenbeck 1967
	30.0	28.0	Ivy 1952

It should be emphasized, however, that these are pressure obtained during obstruction and are not true resting pressures. Values obtained when the ducts are not obstructed give different results (see later).

Elliott and colleagues (1957) determined the pressures in the bile and pancreatic ducts of unanaesthetized dogs following prolonged obstruction. The secretory pressure of the pancreas was initially about 30 cm H_2O. Following ductal obstruction it rose uniformly, but within 24 hours it fell again and approached its initial value. The biliary pressure increased steadily to 25-30 cm H_2O and exceeded the pancreatic pressure after 12-24 hours of obstruction.

Cholangiomanometry

This technique was developed to provide a means of detecting any

obstruction to the major bile ducts and to confirm that there is no hindrance to the normal flow of bile (Daniel 1972, Caroli 1946). Cholangiomanometry is a method of determining the resistance of the sphincter of Oddi to the free flow of bile into the duodenum as it has been suggested that choledochal pressures are a product of sphincteric activity (Cuschieri 1972). The highest normal opening and resting pressures in man are considered to be 15-16 cm H_2O (White 1978, Daniel 1972, Doubilet 1937, Kraus 1967, McCarthy 1970, Schein 1970). In the presence of pathology in the ducts the opening pressure rises above 16 cm H_2O to values in excess of 30 cm H_2O (White 1978, Daniel 1972). Cuschieri (1972) has demonstrated pancreatic duct reflux during manometric choledochography at pressures below the sphincter opening pressure. Schien (1968) studied choledochal dynamics in man using cinemanometric cholangiography, He found that the average pressure required for pancreatic duct filling was 23 cm H_2O (15-40 cm H_2O) and that this was not related to choledochal pressure.

These observations suggest that there is a sphincter opening pressure of 15 cm H_2O or above when gallstones are present in the common bile duct. Pancreatic duct reflux can occur when pressures in the bile duct are well within the physiological range.

Endoscopic manometry

The recent advent of endoscopic retrograde cholangiopancreatography (ERCP) has allowed non-operative measurement of pressures in the human pancreatic and bile ducts (Ribiero 1977, Csendes 1979, Carr-Locke 1981). Csendes (1979) measured resting pressures in the common bile duct to be

11.4±1.3 mm Hg in controls, 12.8±0.9 in those with gallstones and 8.9±1.9 in those with choledochal stones. The pressures in the pancreatic duct for the corresponding 3 groups of patients were 32.5±3.3, 28.4±1.9 and 25.3±1.9 mm Hg. A review of pressures measured by endoscopic manometry in the normal resting biliary and pancreatic ducts is given below.

	choledochal pressure (mmHg)	pancreatic duct pressure (mmHg)
Csendes 1979	11.4±1.3	32.5±3.3
Hogan 1977a	11	—
Hogan 1977b	13	—
Carr-Locke 1981	3.0±2.5	11.4±3.0
Rosch 1976	8.8±2.1	10.9±3.1
Geenen 1977	9.3±2.0	—
Bar-Meir 1979	12.0±1.0	15.0±1.0
Anacker 1977	12.0±3.5	22±7.3

(N.B. Different catheter size, infusion rate and compliance makes results not strictly comparable).

Tanaka (1981) demonstrated ductal pressures to vary with respiration and posture changes as the pressures rose on inspiration and in the prone position. The values obtained by these investigators, while not comparable, indicate that in the normal fasting patient the pressure in the pancreatic duct is greater than that in the common bile duct.

Sphincter of Oddi motility

Recent developments in instrumentation have enabled an increased

understanding of sphincter of Oddi physiology (Toouli 1984). Prior to the advent of endoscopic manometry most of the studies of sphincter of Oddi function had been performed in experimental animals, in man during surgery on the biliary tract (Schein 1970) and patients with T tubes (Bergh 1942) in the biliary tract. Studies in a variety of experimental animals (cats, dogs, opossums, monkeys) have demonstrated that the sphincter of Oddi (SO) functions independently of duodenal muscle activity (Toouli 1984, La Morte 1980, Becker 1982). The predominant mechanism of common bile duct emptying in the opossum is the antegrade SO phasic contraction (Toouli 1983) and the caudal 3-4mm of the sphincter has a narrow lumen with a basal pressure of 15 mm Hg. The common bile duct and pancreatic duct proximal to the sphincter do not demonstrate spontaneous motor activity, and appear only to act as compliant capacitance conduits. Thus during the fasting state control of bile flow is maintained by prominent phasic contractions that continually expel small volumes of bile into the duodenum. These contractions are inhibited by cholecystokinin or CCK (Behar 1980).

Manometric studies in man have demonstrated a high pressure zone corresponding to the SO, with phasic pressure changes superimposed on the basal sphincter pressure (Csendes 1979, Carr-Locke 1981). A recent study (Toouli 1982a) showed that the SO was characterized manometrically by prominent phasic contractions superimposed on a basal SO pressure 3 mm Hg above the pressure in the common bile duct and pancreatic duct. The amplitude of the phasic contractions was 130 mm Hg and their frequency 4 per minute. The majority of contractions were antegrade (60%) although some contractions occurred simultaneously (25%) and 15% were retrograde in action. CCK inhibited basal contractions and produced

a fall in the basal SO pressure (Toouli 1982b). It was concluded from these studies that, during fasting, the human SO exhibits peristaltic type phasic contractions which propel bile into the duodenum and prevent reflux of duodenal contents into the bile, and pancreatic ducts. During feeding (CCK released) passive movement of bile occurs across the SO into the duodenum.

Several sphincter of Oddi motility disorders have now been described, although the evidence for their existence remains sparse (Toouli 1984). Spasm of the SO is characterised by an elevation of SO basal pressure - producing increasing resistance to bile flow from the common bile duct into the duodenum. Some patients appear to have a paradoxical response to CCK injection with an increase in SO pressure (Hogan 1982). Other patients have an increased frequency of contractions of the SO causing an obstruction to bile flow, similar to morphine action (Berci 1965, Toouli 1984).

A recent study has described abnormalities in the orientation of SO contractions (Toouli 1982a) in patients with choledochal stones. In these patients there is a significant decrease in the percentage of contractions orientated in an antegrade direction. It was, however, uncertain whether the abnormal retrograde contractions preceeded stone formation or were due to the presence of stones themselves. As gallstone pancreatitis has been attributed to gallstone migration down the biliary tree and through the SO, (Acosta 1974, Kelly 1976) it is possible that an abnormality of SO motility might lead to bile reflux into the pancreatic duct.

These preliminary human studies suggest that motility disorders of the SO may be partly responsible for the initiation of reflux into the pancreatic duct. The SO pressures measured in man are certainly well above those in both the bile and pancreatic duct indicating that reflux between the two ducts is likely during episodes of SO contraction.

Pressure relationships

As fluid flows down hydrostatic pressure gradients a knowledge of pressure relationships within the pancreatic and biliary ducts and duodenum is important. These pressures are direct pressures and are not the maximum secretory pressures mentioned previously.

Biliary pressure

Intraluminal pressures of the common bile duct were first measured in dogs through a T-tube with a mean value of 12 cm H_2O (Potter 1926). Parry and associates (1955) and Menguy and co-workers (1958) introduced polyethylene tubes into the common bile ducts and pancreatic ducts of dogs, pulling the tubes through the parenchymata of the organ until the open ends lay in the mid-portions of the ducts well away from the sphincters, which were free to function normally. Parry (1955) found common bile duct pressures to measure 4.8 cm H_2O (0.3-9.4) in the resting state and Menguy (1958) found it to be 7.1 cm H_2O (0-15.8). In humans the common bile duct pressure has been measured via a T-tube at 6-29 cm H_2O, the majority of readings being between 10 and 15 cm H_2O (Jacobson 1957, Anderson 1962, Bergh 1942).

In the biliary tract the gallbladder serves as a pressure regulator (Hansson 1967), and the pressure varies with the state of contraction of

the gallbladder. After removal of the gallbladder the pressures in the bile duct during fasting were on average 4 cm H_2O higher when measured in dogs (Menguy 1958, Hansson 1967). Feeding produces a marked rise in choledochal pressure with pressures rising to above 30 cm H_2O (Hansson 1967) about 30 minutes after the beginning of the meal. However, it should be noted that the degree and rate of increase in choledochal pressure varies considerably from animal to animal (Hallenbeck 1967, Parry 1955). Hormonal stimulation by secretin and CCK produces an increase in biliary pressures to values of 30 cm H_2O (Hansson 1967). This increase in pressure while less dramatic than that produced in the pancreatic ducts, is of longer duration. Several analgesic derivatives of morphine increase the sphincter of Oddi pressure with a subsequent rise in choledochal pressure (Scendes 1979, Hopton 1967).

Pancreatic pressure

An increase in pressure within the pancreatic duct system has been claimed to be an aetiological mechanism in the genesis of the two commonest forms of acute pancreatitis, alcoholic and gallstone (Klein 1983a,b). This increase in pressure may be due to an increase in pancreatic exocrine secretion simultaneous with ductal obstruction (Banks 1971, Gambill 1973) or following free reflux of biliary and duodenal fluid (Acosta 1974, McCutcheon 1968). The dynamics of pancreatic ductal pressure changes is thus very important for a complete understanding of the pathophysiology of pancreatic disease.

Canine pancreatic duct pressure was initially measured by Parry and associates (1955) to be 9.1 cm H_2O (range 6.8-13.1) and by Menguy (1958) to be 14 cm H_2O (3.5-29.3). Gilsdorf (1967) determined the average

pancreatic ductal pressure to be 18.3 cm H_2O (11-27) and the mean bile duct pressure to be 17.6 cm H_2O (7-25). Dimagno and colleagues have measured the fasting pancreatic duct pressures in unanaesthetized dogs and found them to be 29.8±0.8 cm H_2O (Owyang 1977) and 23 cm H_2O (Dimagno 1979).

Fewer studies have been performed for measurement of intrapancreatic duct pressure in humans (Anderson 1962, White 1970, 1964, Duval 1958). Values ranging from 10 to 30 cm H_2O are reported in these studies with a mean value of 20-25 cm H_2O. Stimulation of pancreatic secretion by feeding (Hallenbeck 1967) or secretin/CCK (Hansson 1967) produces a marked increase in pancreatic duct pressure. This increase however is less sustained than that seen in the bile ducts. White and colleagues (1964) reported that pancreatic intraductal pressure increased from a range of 7-12 cm H_2O to as much as 30 cm H_2O after feeding and that secretin produced pressures as great as 38 cm H_2O.

Klein (1983a,b) has carefully investigated regulatory factors of pancreatic intraductal pressure. In experiments using dogs he demonstrated basal pancreatic pressure to be 11.2±1.0 cm H_2O. This pressure was increased by gastric alcohol to 17.7±1.6 cm H_2O, and reduced by duodenal alcohol to 5.5±0.9 cm H_2O. Vagotomy produced a 40% reduction in ductal pressure. Secretin produced an 80% increase in pressure, this increase was less marked after vagotomy. These results demonstrated the vagus nerve to have an important role in maintaining pancreatic intraduct pressure. Vagotomy reduced pancreatic secretion and relaxed the sphincter and duct wall. Further studies from the same laboratory (Klein 1973b) suggested that the autonomic nervous system affected pancreatic intraductal pressure i.e. vagal stimulation increased pressure, vagotomy decreased pressure.

Although considerable controversy exists regarding the role played by the sympathetic system (Singh 1978, Dressel 1979), Gilsdorf et al (1967) have demonstrated increased pancreatic intraductal pressure during sympathetic nervous system stimulation. Furthermore, coeliac plexus denervation produces a significant pressure decrease in the canine pancreatic ducts (Klein 1983b). It has also been suggested that local reflex arcs play a significant role in controlling pancreatic pressures (Tiscornia 1976). These observations indicate that the pancreatic ductal pressure depends on at least three factors (i) rate of secretion, (ii) activity of the sphincter mechanism and (iii) tone of the duct itself.

A summary of factors involved in the regulation of pancreatic intraductal pressure is given below.

pancreatic intraductal pressure

increased	decreased
feeding	vagotomy
CCK	coeliac plexus denervation
secretin	duodenal alcohol
vagal stimulation	atropine
sympathetic stimulation	
hypothalamic stimulation	
gastric alcohol	
↑ neural tone in local arcs	
opiates	

Duodenal pressure

Pressures in the duodenum were measured during operation by Daniel (1972) and found to be approximately 10 cm H_2O and during ERCP by Anacker (1977) with values of 5.3±2.7 cm H_2O. Dimagno and colleagues have extensively investigated duodenal pressures in the unanaesthetized dog. They found the fasting duodenal pressure to vary between 22.9±1.12 cm H_2O (Owyang 1977), 8±2 cm H_2O (Dimagno 1981a) and 18.4±1.9 cm H_2O (Dimagno 1979). After feeding the duodenal pressure increased and reached a peak of 48.5±0.5 cm H_2O at 45 minutes. This postprandial increase was postulated to be a result of both a neural (Bozler 1949, Kosterlitz 1968) and a hormonal stimulation by CCK (Hedner 1967, Harvey 1975). Secretin produced a marginal reduction in duodenal pressure (Dimagno 1981).

Abdominal pressure

Little is known of the factors influencing intra-abdominal pressure or its importance in surgical pathology of the abdomen. Gedda (1983) studied pressures in the subhepatic space after biliary surgery and found that the pressure was influenced by breathing and position. Normal breathing was associated with an intra-abdominal pressure of 2.5 mm Hg, deep breathing a pressure of 5.0 mm Hg and coughing 24.0 mm Hg. These pressures were increased in the erect position with maximum values obtained of normal breathing 5.8 mm Hg, deep breathing 11.3 mm Hg and coughing 60.8 mm Hg. These findings are in keeping with the earlier results of Kewenter and co-workers (1969). Indeed Ivy (1952) and Mann and Giordano (1923) have measured pressures in the common bile duct of greater than 100 cm H_2O during vomiting, coughing or straining. Thus duct and duodenal pressures may be modified by pressures in the subhepatic space. As the bile duct is an intraperitoneal structure throughout much of its course it is likely

that the biliary pressures could be more directly influenced by intra-abdominal pressure than the retroperitoneal duodenum and pancreatic duct. Vomiting and increased intra-abdominal pressures (Dragstedt 1934) are associated with biliary colic and may thus be important in the context of reflux into the pancreatic duct.

More important than static pressure measurements are the dynamics of pancreatico-biliary-duodenal pressure gradients, which may well be important for a complete understanding of pancreatic disease (Klein 1983a,b). Without analysis of these dynamic events it is easy to dismiss reflux into the pancreatic duct as being of little importance, since, in most instances, the mean pressure in the pancreatic ducts is greater than that in the biliary system and duodenum. Pressure differentials within the duct-duodenal system may produce reflux, with the amount and depth of reflux being dependent on the actual pressure difference.

Biliary-Pancreatic reflux

Although the pressure in the pancreatic duct is usually greater than that in the biliary tree (Hallenbeck 1967) bile refluxes at times into the pancreatic duct system. Occlusion of the common channel in the rat is associated with bile reflux into the pancreatic ducts (Block 1955). Herriott and colleagues (1966) created a common channel in rats between the bile duct and pancreatic duct, and demonstrated bile to pass into the pancreas. Indeed it appears that bile duct pressures may increase above those in the pancreatic duct after obstruction of the common channel (Herriott 1966, Hansson 1967), after cholecystectomy (Hansson 1967), after administration of cholecystokinin (Gamklou 1966) and occasionally after

feeding (Menguy 1958, Parry 1955). Gamklou (1966) occluded the common channel in rats and studied the effects of the hormones CCK and secretin. The administration of CCK stimulated bile flow and gross reflux of bile into the pancreas. Secretin, however, prevented bile reflux as a consequence of increased pancreatic flow. A recent study by Becker and associates (1979) summed up thoughts on bile reflux thus: "The possibility of reflux has been explored in numerous experimental models. Nevertheless, uncertainty exists regarding the frequency and quantity of biliary pancreatic reflux under physiological conditions (Hicken 1952, McCutcheon 1968), its mechanism of production (Caroli 1960), the physiological and pharmacological factors influencing it, as well as its pathological significance (Schiller 1974). This uncertainty is due in part to the fact that these models have suffered from the abnormal pressures in the system". Becker and co-workers (1979) developed an elegant primate model for studying biliary pancreatic reflux by inserting cannulas into the gallbladder and common bile duct of rhesus monkeys with creation of a pancreaticocutaneous fistula. Hypaque was instilled into the gall bladder with maintenance of common duct pressures within a normal range. The pancreatic duct was visualized in 62% of radiographic studies and small amounts of iodine were detected in the fistula effluent. Further studies using ^{14}C - PEG demonstrated unequivocal reflux into the pancreatic duct system. Furthermore they demonstrated reflux to occur while the pressure at the distal end of the pancreas was well above common bile duct pressure, reinforcing the concept of dynamic pressure gradients. Becker's study is an important one as he demonstrated for the first time biliary pancreatic reflux to occur under relatively physiological conditions. The finding of pancreatic duct reflux on operative cholangiography adds further credence to the theory of bile reflux

(Taylor 1981, Kelly 1976, Cuschieri 1972) (see later).

Duodeno-pancreatic reflux

An understanding of the dynamic relationship between duodenal and pancreatic pressures is vital if duodeno-pancreatic reflux is of importance in the pathogenesis of acute gallstone pancreatitis. This theory was revived by McCutcheon (1968) after the earlier development of closed loop experimental models of pancreatitis (Pfeffer 1957). Although Rosato and co-workers (1970) indicated that in the closed duodenal loop situation an elevated intraduodenal pressure alone did not produce pancreatitis, Johnson and Doppman (1967) demonstrated that hormonal stimulation with secretin and CCK in the presence of duodenal distension did cause transampullary reflux of duodenal contents in the rhesus monkey.

An elegant study in dogs (using microspheres) by Johnson and colleagues (1976) demonstrated definite duodeno-pancreatic reflux to occur and when duodenal pressure was increased by retching or ethanol administration the frequency of reflux was elevated. Dimagno and associates (Owyang 1977, Dimagno 1979, Hendricks 1980, Keane 1981, Dimagno 1981) demonstrated that in dogs mean fasting pancreatic pressure was 5 to 10 cm H_2O higher than mean fasting duodenal pressure. However, after a meal, both pressures increased and the mean duodenal pressure was higher than the mean pancreatic pressure 20 minutes after feeding. They further studied duodenal reflux into the pancreatic duct using ^{14}C - PEG and demonstrated reflux to occur more frequently after feeding than fasting (38% vs. 10.8%). The total volume of duodenal content refluxed represented between 0.5% and 1% of total pancreatic volume flow and between 0.05%

and 0.07% of total duodenal volume flow.

Despite the static pressure gradients, definite bile and duodenal reflux into the pancreatic duct can occur. The importance of this reflux is dependent on the nature of the fluid, its pressure and volume, and the nature of the pancreatic host defences.

The effect of pressure on the pancreasA common factor of the various theories for the pathogenesis of acute gallstone pancreatitis is that of increased pressure within the pancreatic ducts leads to escape of duct contents into the interstitial tissues. Previous investigators have studied the effects of pressure on the pancreatic ductal system. Rich and Duff (1936) were pioneers in this field. They injected (no pressure measurement) Indian ink into the pancreatic duct of dogs and produced ruptured acini with extravasation of Indian ink into the interstitial tissues. Rupture of small ducts and leakage through clefts between the aciner cells appeared to be responsible. Egdahl (1958) injected Indian ink into the pancreatic duct of dogs with more attention to pressure. At a pressure of 20-40 cm H_2O there was no evidence of duct rupture but there was passage through clefts between acinar cells out into the interstitium of the gland. At higher pressures of 200 cm H_2O duct rupture was routine. Edlund and colleagues (1963) used thorotrast to determine the effect of intraductal pressure increase on the rat pancreas. They demonstrated that thorotrast particles pass from the lumina of the glands via intercellular spaces to the pericapillary space. Duprez et al (1963) reported similar results following retrograde injections of fluorescein dye into the pancreatic duct. Mallet-Guy (1958) described extravasation of dye into periacinar tissue when it was infused into the pancreatic duct at pressures between 35 and 37 cm H_2O. Herriott and Palmer (1966) showed

that Indian ink extravasation in rats could occur as a result of biliary pressure alone and was associated with evidence of small duct ruptures. Pirola and Davis (1970) closely studied the effect of pressure on the integrity of the duct-acinar system of the cat pancreas. They carefully correlated pressure with the microscopic and macroscopic changes produced by Indian ink. When a pressure of 20 cm H_2O was used there was no evidence of ductal extravasation. With 30 cm of pressure there was evidence of mild extravasation through intercellular clefts. At 40 cm H_2O the pancreas was densely stained black with evidence of gross extravasation through duct ruptures. They concluded that duct extravasation could occur at pressures below the maximum secretory pressures of the pancreas.

Bockman and co-workers (1971) used retrograde infusions of ferritin into the canine pancreatic duct at carefully controlled pressures, and observed the distribution of the injected agent by the use of both light and electron microscopic techniques. They reported accumulation of ferritin in a periacinar space. Access to this space appeared to occur through minute disruptions of the cellular lining at the ducto-acinar junction with the infusion mixture passing into a potential space between the plasma membrane of the acinar cell and the basal lamina. In a study by Anderson and Schiller (1968), dilute solutions of Indian ink were infused into the pancreatic duct at pressures of 50 to 100 cm H_2O. Light microscopy revealed that ink particles were present between acinar cells and concentrated at the basal aspect of acinar units. These findings were interpreted as evidence that ink passed from the ductal lumen between the acinar cells and into a space at the base of the acinus. The "periacinar spaces" appeared to be intimately associated

with the pancreatic lymphatic and capillary system, and the ink particles were rapidly cleared from the normal canine pancreas. Thus extravasated saline or Indian ink passes into the periacinar and interstitial spaces before being rapidly cleared by the capillary and lymphatic system (Schiller 1972).

Summarising the results of these studies reveals that at:

- : low pressures (20 cm H_2O) no extravasation occurs
- : moderate pressures (20-30 cm H_2O) extravasation occurs via intercellular clefts
- : high pressures ($>$ 35 cm H_2O) duct rupture occurs.

Pancreatic intraductal fluid can thus reach the interstitial tissues in the absence of ductal rupture or of high intraductal pressures by sifting through clefts between acinar tissues (Gambill 1973, Banks 1971). The effect of injection of fluids into the pancreatic ducts is therefore very dependent on the pressure of injection and a comparison between the results of such injections is invalid unless careful consideration be given to pressure.

Pressure and ERCP

The recent development of ERCP as a radiological tool for the investigation of pancreatic duct structure and physiology has highlighted the importance of volume and pressure of injection. Hermann (1979) and White (1975) state that 1.5-2.0 ml of dye is all that is required for filling of the pancreatic duct with 5 ml being the maximum volume. They further emphasize that overfilling of the pancreatic duct system may induce pancreatitis. Anacker (1977) has correlated the degree of duct filling (i.e. volume and pressure) with

elevations in serum amylase and lipase. The greatest changes in these enzymes were associated with parenchymal filling during ERCP examination. Recent experience in our unit emphasizes these points. A 52 year old lady underwent ERCP examination for suspected pancreatic disease. 3 mls of contrast were infused into the pancreatic duct at a high pressure with the x-ray film illustrated in fig. 8. This demonstrates a parenchymogram (acinar opacification) with probable duct extravasation in the head of the gland. Following this examination the patient developed severe abdominal pain with a grossly raised serum amylase (26,000 u/l). We are certain that this patient developed acute pancreatitis secondary to high pressure rupture of the pancreatic ducts. Fortunately, she recovered fully, but it remains debateable whether this relatively benign course would have followed if the extravasated fluid was toxic and not the relatively bland constrast medium.

Kasugai et al (1974) investigated pressure and ERCP examinations and demonstrated a correlation between the infusion pressure and raised amylase levels. The lowest pressure at which amylase will pass into the blood in man is known to be less than 30 cm H_2O (Ivy 1952). Howell and Bergh (1950) observed that when, during the course of T-tube cholangiography, the radio opaque material or bile passed into the pancreatic duct under a pressure of 20-42 cm H_2O, a rise of serum amylase occurred within 30 minutes. Bilbao (1972) lists the factors that are associated with ERCP-induced pancreatitis as; speed and pressure of injection of contrast material, the volume injected and the number of injections into the pancreatic duct. The use of large volumes tend to overdistend the pancreatic ducts and cause acinar opacification and there is evidence that post-injection pancreatitis occurs almost exclusively amongst patients

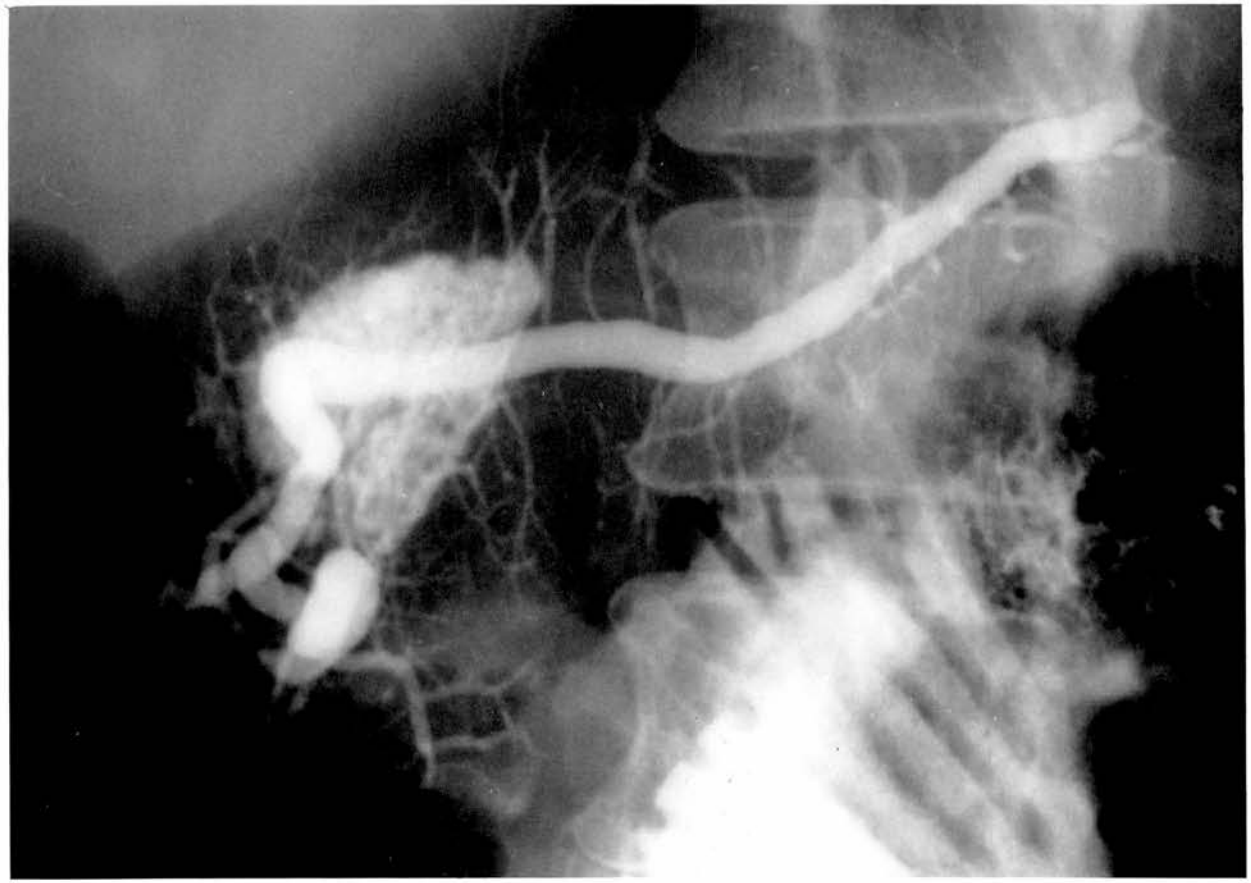


Fig. 8 ERCP radiograph. Note acinar filling and duct rupture in head of gland.

with acinar opacification (Bilbao 1976). The incidence of post-injection pancreatitis can be reduced considerably by using small amounts of contrast material at physiologic pressures and utilising careful fluoroscopic monitoring of the effects of injection. In one study (Kasugai 1974), with manometric control of injection pressure, the frequency of acinar filling was reduced to 2.4% and there was no clinical evidence of acute pancreatitis. The reverse also appears to be important as White (1975) has demonstrated that a recent attack of pancreatitis is associated with diffuse opacification of the pancreas after only a small volume of dye is injected. These reports reinforce the importance of duct pressure in producing pancreatic damage.

Pressure and pancreatitis

This will be discussed more fully in the next chapter. Briefly, however, bile at low pressures produces no pancreatic damage (Robinson 1963, White 1960) whereas the same bile at high pressures (Davenport 1976, Banks 1971, Sum 1970, Gilsdorf 1967, Gamklou 1966, Blumenberg and Powers 1963) will induce acute pancreatitis. These observations, while requiring further elucidation, demonstrate a definite relationship between pressure, bile and pancreatitis. Indeed Guelrud (1984) has stated; "the theory that canalicular hyperpressure appears to be a major factor in producing acute pancreatitis is supported by the experimental reproduction of the disease".

These varied observations, stimulated me to examine the effect of pressure alone on the pancreas before proceeding to examine the effect of more toxic substances.

Materials and methods

object: To investigate the effect of pressure alone on the pancreas before proceeding to evaluate more toxic substances. The pressures studied were those known to occur in the bile-pancreatic-duodenal system. It is unlikely that the maximum pressure exerted (50 cm H_2O) is surpassed in the physiological situation.

experimental preparation: The experimental preparation described in figure 5, was used in this study. The volume of 50 µl evaluated in the previous chapter was used in all studies. The injectate was 0.9% sterile saline. 50 µl of saline was infused into the pancreatic ductal system at varying rates to produce maximum pressures of 10, 15, 20, 25 and 50 cm H_2O. The low pressures required a longer period of infusion to deliver a given volume. (i.e. 10 cm H_2O - 5½ mins, 15 cm H_2O - 3 mins, 20 cm H_2O - 1 min, 25 cm H_2O - 30 secs, 50 cm H_2O - 3 secs). Following delivery of 50 µl saline the infusion pump was discontinued and the cannula left in situ for a short (5 minutes) or long (60 minutes) period. During these periods the pressure in the system fell as indicated in figures 9 and 10.

After the set period of occlusion (short or long) the cannula and ligature were removed, the duodenum was closed with a single 7/0 prolene suture and the abdomen in layers. The animals made a full recovery with free access to food and water until sacrifice at 24 hours. Pancreatic damage was assessed as previously described in Chapter II. Two groups of animals were used for comparative

purposes: laparotomy only (sham) and cannulation only (control).

Pancreatic and biliary pressures in rats

Direct measurement of the corresponding pancreatic and biliary pressures was performed in 10 anaesthetized rats. A biliary cannula was inserted into the bile duct close to the liver. A pancreatic cannula was inserted into the isolated BPD. The results of these measurements were

biliary pressure, median 8 cm H_2O, range 5.14

pancreatic pre-sure, median 11 cm H_2O, range 6-21

At most measurements (82%) the biliary pressure was below that of the pancreas. In some (18%) recordings the biliary pressure exceeded pancreatic pressure. When the two cannula were joined (i.e. free passage between bile and pancreatic juice) there was little movement for several minutes. After 5 minutes, however, there was often movement of bile into the pancreas down a pressure gradient.

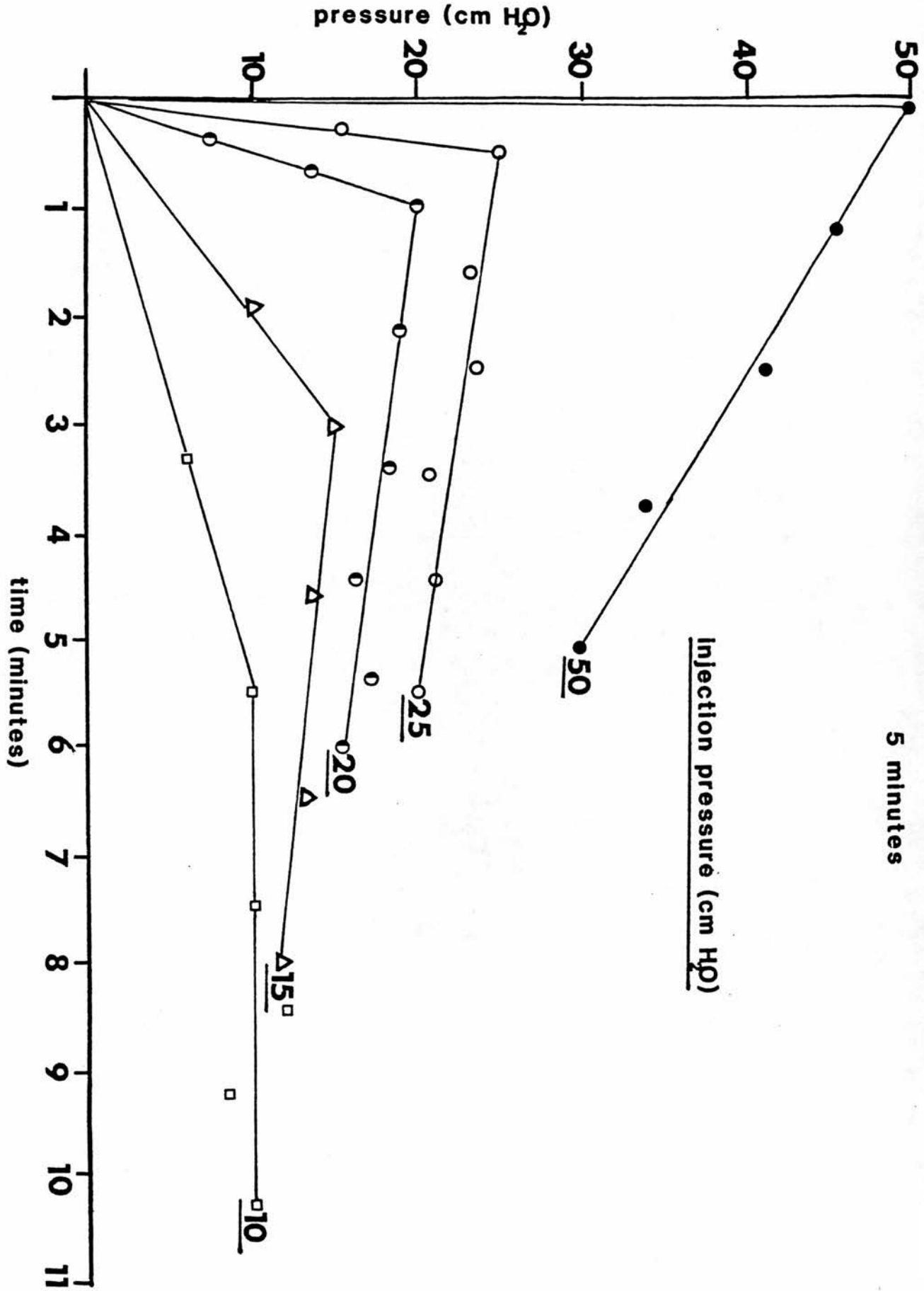


Fig. 9 Pressure fall during 5 minutes occlusion.

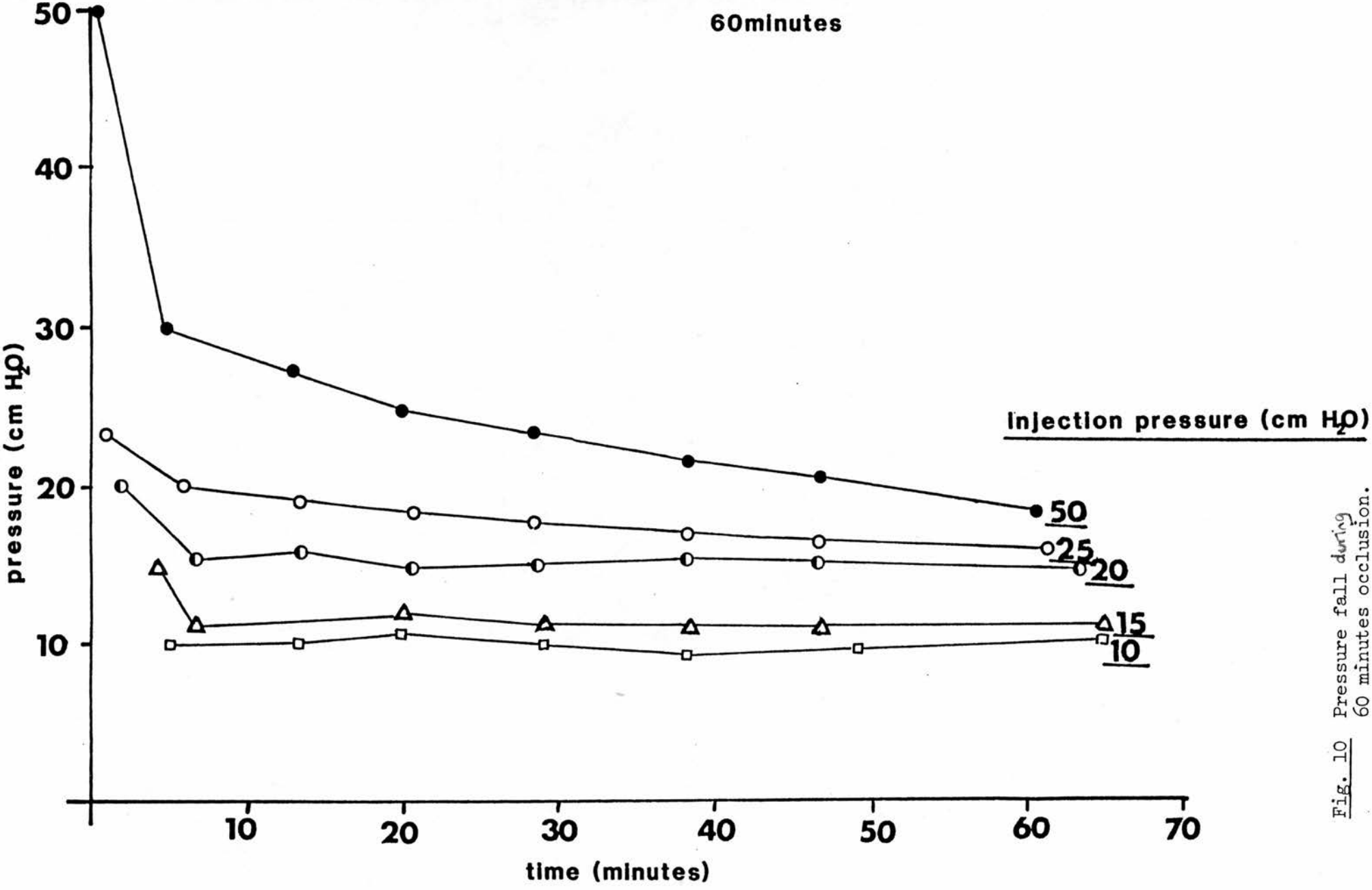


Fig. 10 Pressure fall during 60 minutes occlusion.

Results

All animals survived to 24 hours and no animal developed more than mild pancreatic damage.

macroscopy: There was no evidence of acute pancreatitis as evidenced by the lack of fat necrosis or pancreatic inflammation or haemorrhage. The only change noted was oedema of the gland and a small amount of peritoneal fluid.

pancreatic gland weight ratio (PGWR)

The results for the 5 and 60 minute occlusion times are given in tables 4 and 5 and illustrated in fig. 11.

For the 5 minutes occlusion time there was a significant elevation in the PGWR when the pressure was 25 cm H_2O or above. For the 60 minutes occlusion time there was a significant elevation in the PGWR when the pressure was 15 cm H_2O or above. At corresponding pressures of 15, 20 and 25 cm H_2O the PGWR was significantly greater for the 60 minutes occlusion time than the 5 minutes group. The most marked elevation in the pancreatic gland weight ratio was obtained when a pressure of 50 cm H_2O was applied. At this pressure there was a 40% increase in the PGWR.

water content of gland

The water content of the pancreas at various pressures is illustrated in fig. 12 and the results tabulated in tables 4 and 5. The water content is a good indicator of pancreatic oedema as previously mentioned ($r = 0.91$, $P < 0.001$). Furthermore, the constancy in measured values is evidenced by the low standard

deviations. Cannulation of the duct alone (control animals) produced a moderate increase in water content from 68% to 72%. Five minutes of occlusion produced significant elevations in the water content at pressures of 15 cm H_2O and above. The water content of the pancreas was significantly higher at most pressures in the long occlusion group. Maximum values were obtained with a pressure of 50 cm H_2O and 60 minutes occlusion. Under these conditions the water content was 83%.

serum amylase (SA)

The results for both the 5 and 60 minutes occlusion times are given in tables 4 and 5 and illustrated in fig. 13. Cannulation of the duct alone (control animals) produced a three to four fold elevation in the SA. This illustrates the importance in having both sham and control groups for comparative purposes. In the five minutes group there was a significant elevation in serum amylase at pressures of 15 cm H_2O and above. In the sixty minutes group 50 cm H_2O of pressure produced an elevation in SA. There was little difference between the 5 and 60 minutes occlusion times at any pressure. The results of SA measurements indicate that there is a marked variation between the results of any one pressure as evidenced by the high standard deviation values. This reflects the rather poor correlation ($r = 0.34$, P 0.02) between the degree of pancreatic damage and serum amylase levels which was commented on in Chapter II.

peritoneal fluid amylase (PFA)

This was not detected in animals undergoing laparotomy only (sham).

The volumes measured in the peritoneal cavity were never more than 1-2 mls and often as low as 200 µl. The fluid obtained was generally clear in nature and never haemorrhagic or darkly staining as is associated with acute haemorrhagic pancreatitis (McMahon 1980). The values obtained are tabulated in tables 4 and 5 with fig. 14 illustrating the overall trend (PFA values were less variable than those of SA). Both five and sixty minutes of occlusion gave similar results. There was a significant elevation in PFA at pressures of 15 cm H_2O or above. Maximum elevations were seen at 50 cm H_2O when the PFA was above 30,000 u/l.

Histology

The histological features were predominantly those of oedema (amorphous pink material on H & E stains), occurring both in the inter and intralobular spaces. The most marked changes were seen in the head of the pancreas with a reduction in severity towards the tail of the gland. Figures 15A and B illustrate a normal pancreas gland. Note the normal ducts, blood vessels, islets and acinar tissue. There is no oedema (grade 0). Figures 15C and D demonstrate severe oedema in the inter- and intralobular spaces and there is no evidence of other pancreatic damage. In no animal was there evidence of acinar necrosis or haemorrhage. In a few glands a minimal inflammatory infiltrate was noted with occasional polymorphonuclear leucocytes identified in the interacinar and interlobular spaces. The duct changes identified were those of dilatation with a few duct ruptures observed at high pressures. The histological scores obtained at the various pressures are illustrated in tables 4 and 5 with graphic representation in figs. 16 and 17.

For the 5 minutes occlusion group the histological score rose steadily to a maximum value of 16 (group of 10 animals) at 50 cm H_2O. Virtually the only change observed was oedema although duct dilation and ruptures were identified at the higher pressure (total 16: oedema 10, ducts 5, other changes 1).

The histological appearances in the 60 minute occlusion group were similar although more marked at most pressures. The maximum value of 28 (group of 10 animals) was obtained at 50 cm H_2O. Again oedema was predominant with some duct changes observed at high pressures (total 28: oedema 19, ducts 6, other changes 3). Duct rupture is shown in fig. 18.

It should be noted that the maximum histological score at any pressure or occlusion time was 28 for a group of 10 animals and is in our defined range of mild pancreatic damage. Pressure does not produce acute pancreatitis but appears to be important in the context of ductal extravasation. Figures 19A and 19B diagrammatically illustrate the effect of pressure on the ductal system.

Pressure effects vs. Indian ink studies

The previous Chapter described the effect of pressure on a given volume of 50 µl Indian ink. Correlation with the described 24 hour changes produced by the same pressures is given in table 6. Analysis of these results demonstrates a reasonably good relationship between the known Indian ink extravasation and the pancreatic damage observed at 24 hours.

low pressure: no ductal extravasation and minimal pancreatic damage.

moderate pressure: extravasation through intercellular clefts. Moderate elevations in water content, PGWR and amylase levels with associated mild oedema.

high pressure: extravasation through ductal ruptures. Marked oedema with maximal elevations in water content, PGWR, and amylase levels.

Results summary

- Pressure alone has importance in producing pancreatic damage and must be carefully controlled in meaningful studies on acute gallstone pancreatitis.

- The pancreatic ductal system is a fairly low pressure system capable of withstanding luminal pressures of only 30-40 cm H_2O. Pressures of 20-30 cm H_2O produce leakage and pressures of 30-50 cm H_2O are associated with duct rupture.

TABLE 4 Pancreatic Changes after 5 minutes occlusion (mean ± SD)

						HISTOLOGY			
PRESSURE	NUMBER	PGWR (g/100g)	WATER CONTENT (%)	SA (u/l)	PFA ($u/lx10^3$)	oedema (0-30)	ducts (0-30)	other (0-30)	Total (0-150)
SHAM	5	316±44	68.33±1.16	1119±467	Nil	1	0	0	1
CONTROL	10	329±25	71.44±0.36	3354±1406	4.1±1.31	2	0	0	2
10	10	332±15	* 74.11±2.25	4635±997	5.25±1.26	3	0	0	3
15	10	324±30	72.18±1.38	* 7225±2705	* 8.1±2.79	* 8	1	0	* 9
20	10	341±35	** 73.92±2.34	** 6467±3045	* 9.4±3.2	* 8	1	1	* 10
25	10	** 361±26	*** 78.43±1.78	** 6373±2555	* 9.45±3.31	* 9	2	1	* 12
50	10	** 408±24	*** 79.11±1.77	*** 10295±1443	** 32.4±6.15	* 10	* 5	1	* 16

TABLE 4 LEGEND

All values are for 5 minutes; i.e. pressure v. pressure

PGWR (g/100g)	WATER CONTENT (%)	SA (u/l)
* *P<0.02 v. controls*	* *P<0.01 v. controls*	* *P<0.001 v. controls*
** *P<0.01 v. 25 cm.*	** *P<0.02 v. controls*	** *P<0.001 v. controls*
	*** *P<0.001 v. control*	

PFA	oedema (0-30)	ducts (0-30)
* *P<0.01 v. controls*	* *P<0.02 v. controls*	* *P<0.02 v. controls*
** *P<0.001 v. controls v. 25 cm.*		

Total (0-150)
* *P<0.01 v. controls*

TABLE 5 Pancreatic Changes after 60 minutes occlusion (mean ± SD)

						HISTOLOGY			
PRESSURE	NUMBER	PGWR (g/100g)	WATER CONTENT (%)	SA (u/l)	PFA ($u/lx10^3$)	oedema (0-30)	ducts (0-30)	other (0-30)	Total (0-150)
SHAM	5	319±68	70.37±1.32	1360±510	Nil	2	0	0	2
CONTROL	10	344±11	73.77±1.11	5489±3810	5.15±2.05	8	1	0	9
10	10	318±19	73.64±1.28	3982±2668	6.2±1.78	10	1	1	12
15	10	394±42*	78.23±1.15*	5091±2027	8.4±2.16*	11	2	1	14
20	10	398±23*	77.44±1.23*	4960±1679	10.9±3.28*	11	3	1	15
25	10	396±31*	80.71±1.16**	5489±1511	10.5±4.37*	11	2	2	15
50	10	440±48**	83.10±1.60***	11494±2553*	34.25±5.89**	19*	6*	3	28*

TABLE 5 LEGEND

PGWR (g/100g)	WATER CONTENT (%)	SA (u/l)
* *P<0.001 v. controls*	* *P<0.001 v. controls*	* *P<0.01 v. all others*
** *P<0.05 v. 25 cms*	** *P<0.01 v. 20 cms*	
**	*** *P<0.01 v. 25 cms*	

PFA	oedema (0-30)	ducts (0-30)
* *P<0.01 v. controls*	* *P<0.01 v. controls*	* *P<0.02 v. controls*
** *P<0.001 v. 25 cms*		

Total (0-150)
* *P<0.01 v. controls*

TABLE 5 vs. TABLE 4 LEGEND

Comparison of 60 vs. 5minutes at corresponding pressures.

Significant values given for each test.

PGWR (g/100g)	WATER CONTENT (%)	oedema (0-30)
15 cmH_2O - $P<0.001$	15 cmH_2O - $P<0.001$	50 cmH_2O - $P<0.02$
20 cmH_2O - $P<0.001$	20 cmH_2O - $P<0.01$	
25 cmH_2O - $P<0.02$	25 cmH_2O - $P<0.02$	
	50 cmH_2O - $P<0.01$	

Total (0-150)
50 cmH_2O - $P<0.01$

TABLE 6 The pancreatic effects of pressure and their association with Indian ink extravasation.

Pressure (cm H_2O)	Indian ink	PGWR	chemical oedema	SA	PFA	Histology
Low (10,15)	all in ducts	↑	↑	↑	↑	↑
moderate (20,25)	IC clefts	↑↑	↑↑	↑↑	↑↑	↑↑
High (50)	duct ruptures	↑↑↑	↑↑↑↑	↑↑	↑↑↑	↑↑↑↑

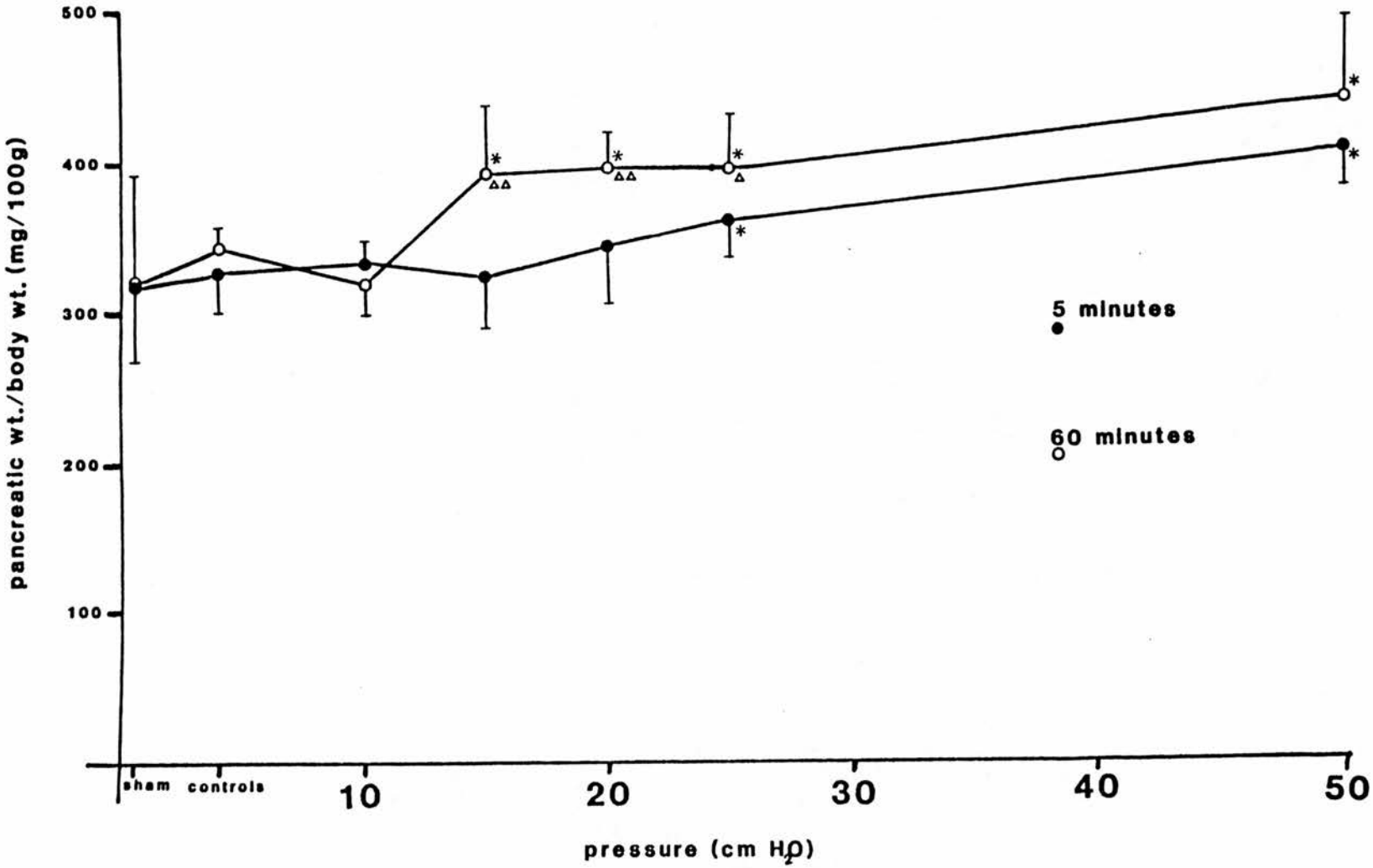


Fig. 11 Pancreatic gland weight ratios vs pressure (N = 10 at each pressure).
* P<0.01 v. controls; Δ P<0.02 v. 5 minutes; ΔΔ P<0.001 v. 5 minutes.

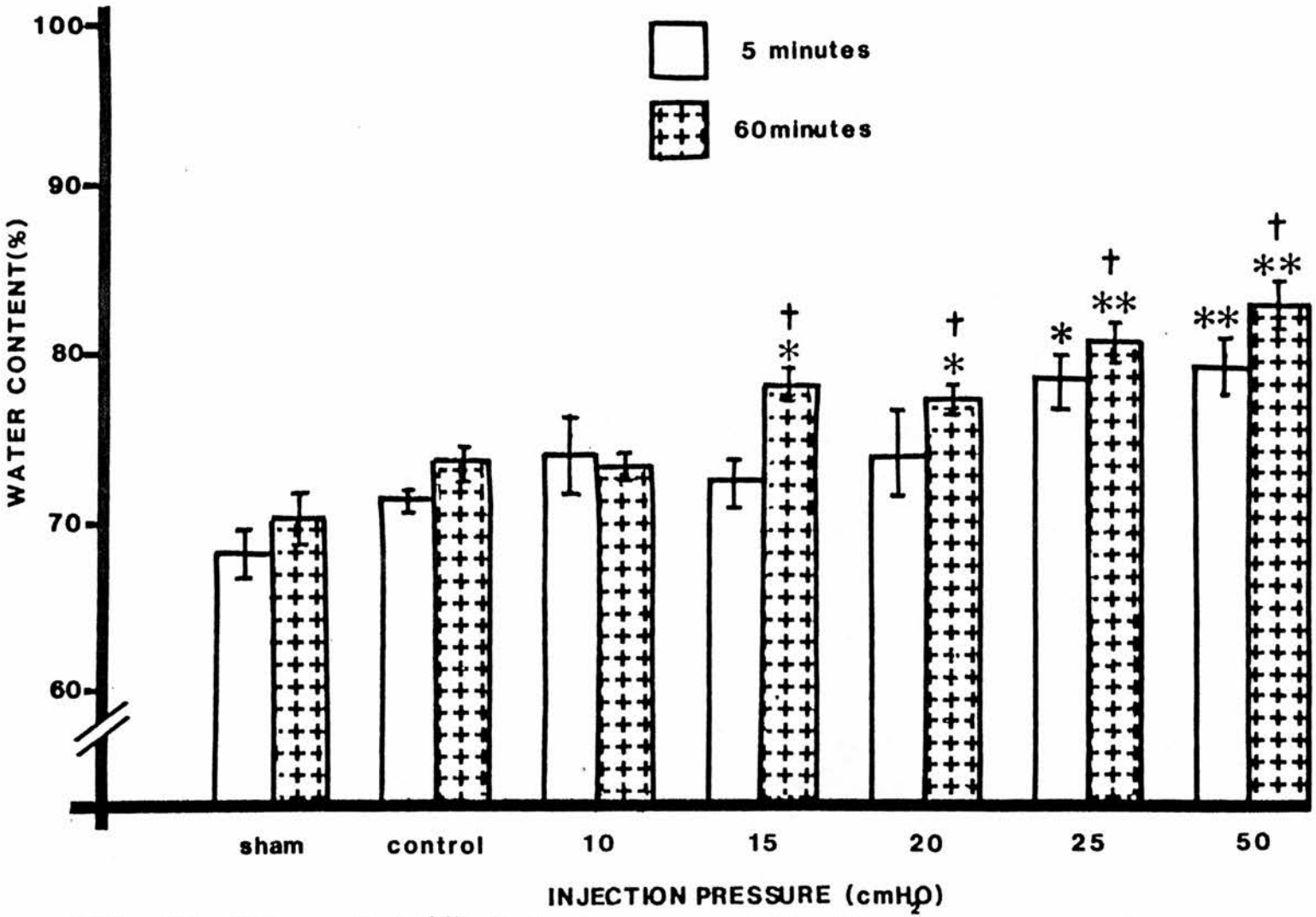


Fig. 12 Water content (%) of gland v. pressure; N = 10 at each pressure. Mean ± SD.
* P<0.01 v. controls; ** P<0.001 v. controls; † P<0.01 v. 5 minutes.

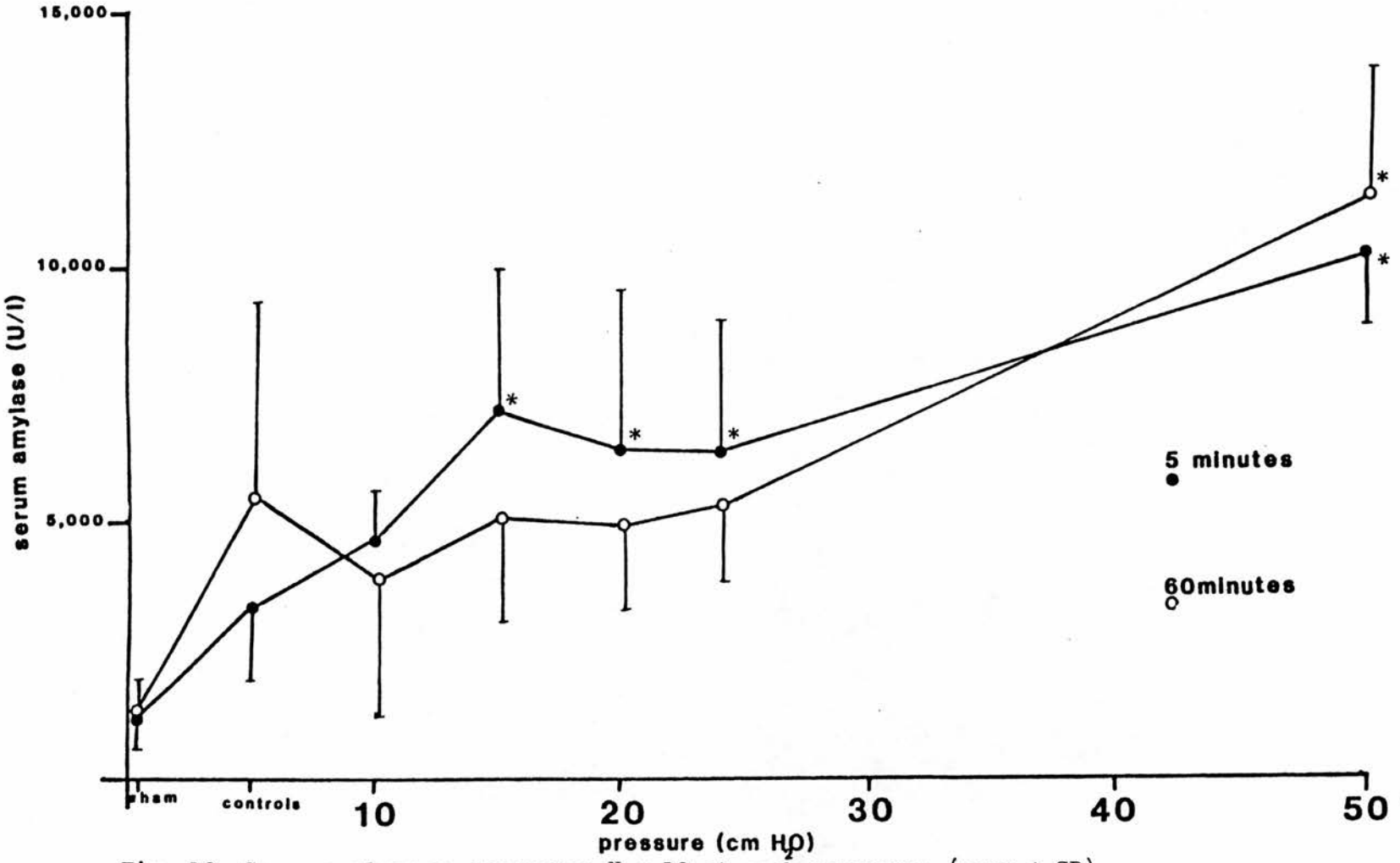


Fig. 13 Serum amylase v. pressure; N = 10 at each pressure: (mean ± SD).
* P<0.01 v. controls.

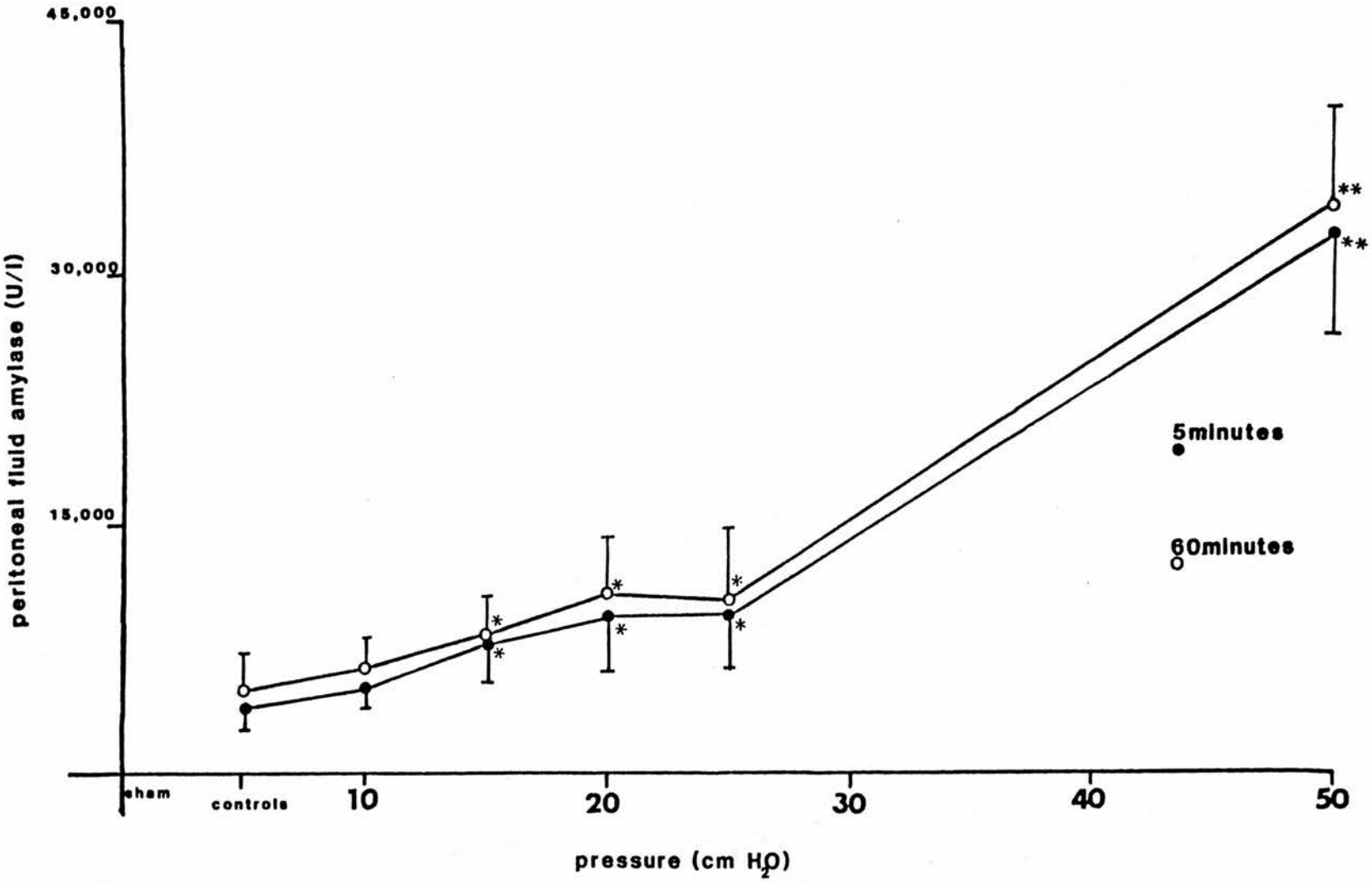


Fig. 14 Peritoneal Fluid Amylase v. pressure; N = 10 at each pressure: (Mean ± SD).
* P<0.01 v. controls; ** P<0.001 v. controls.

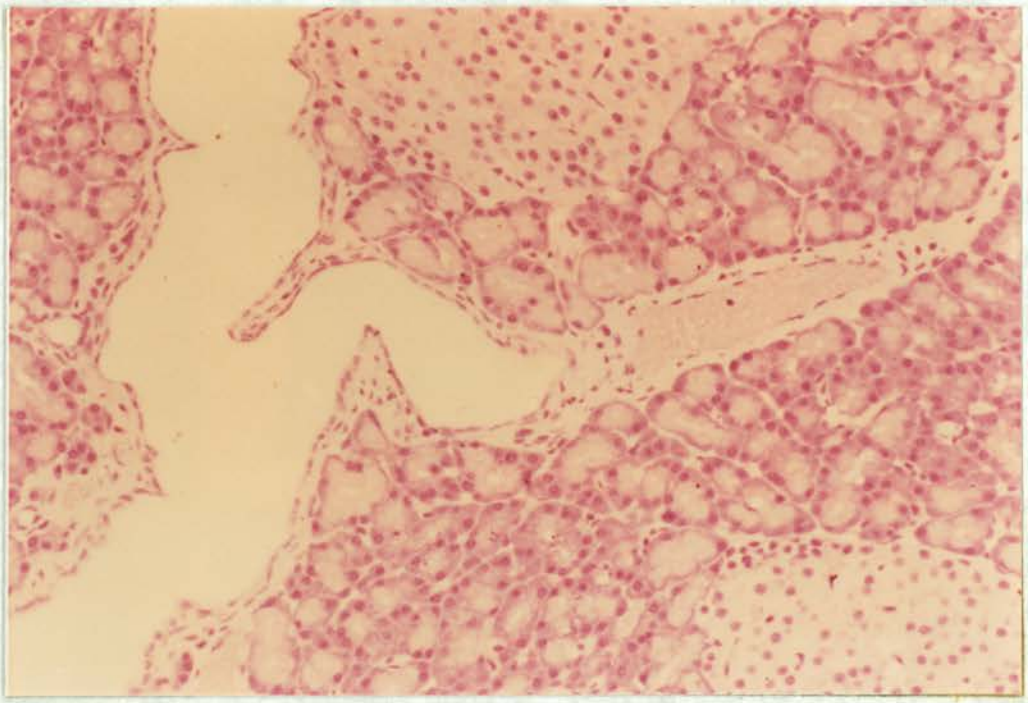


Fig. 15A Normal rat pancreas (x 240).

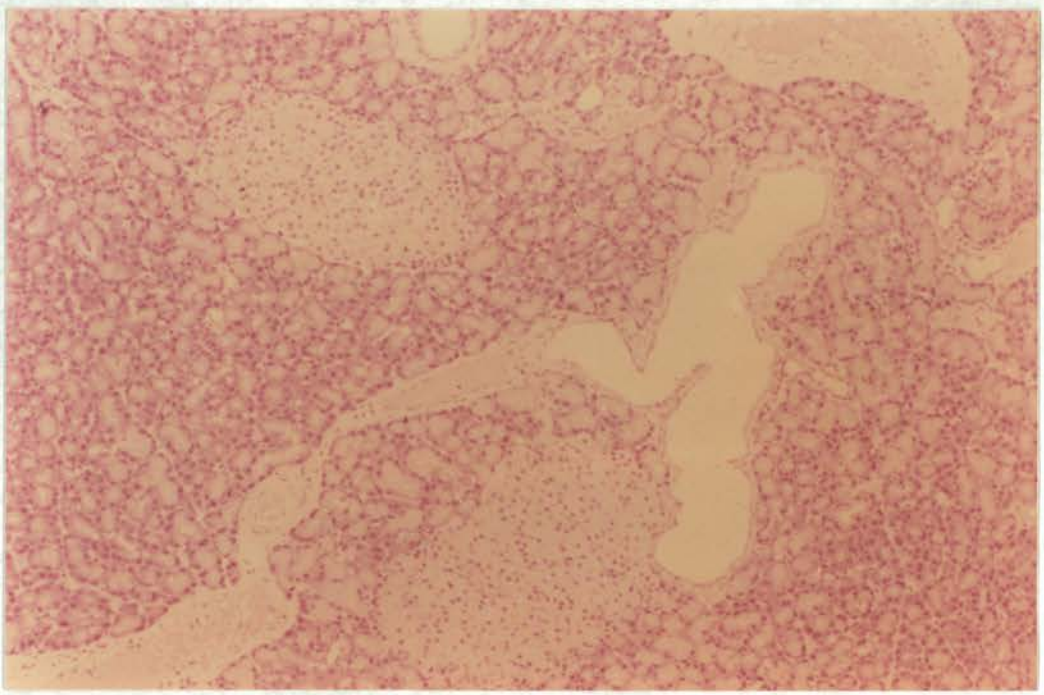


Fig. 15B Normal rat pancreas (x 120).

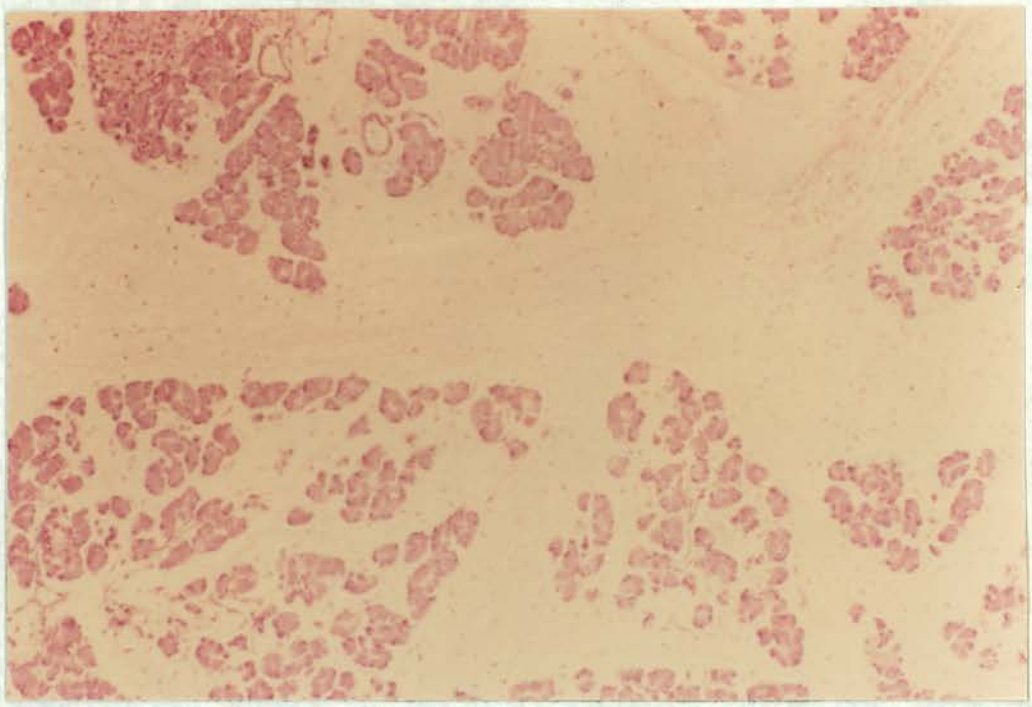


Fig. 15C Severe oedema (x 120).

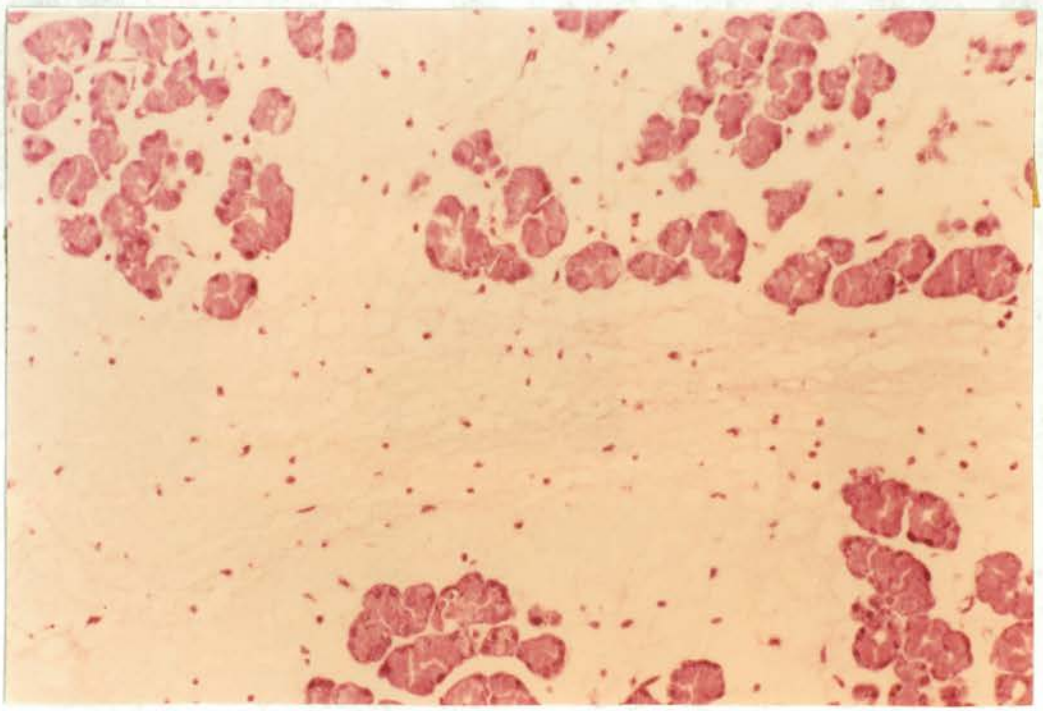


Fig. 15D Severe oedema (x 150).

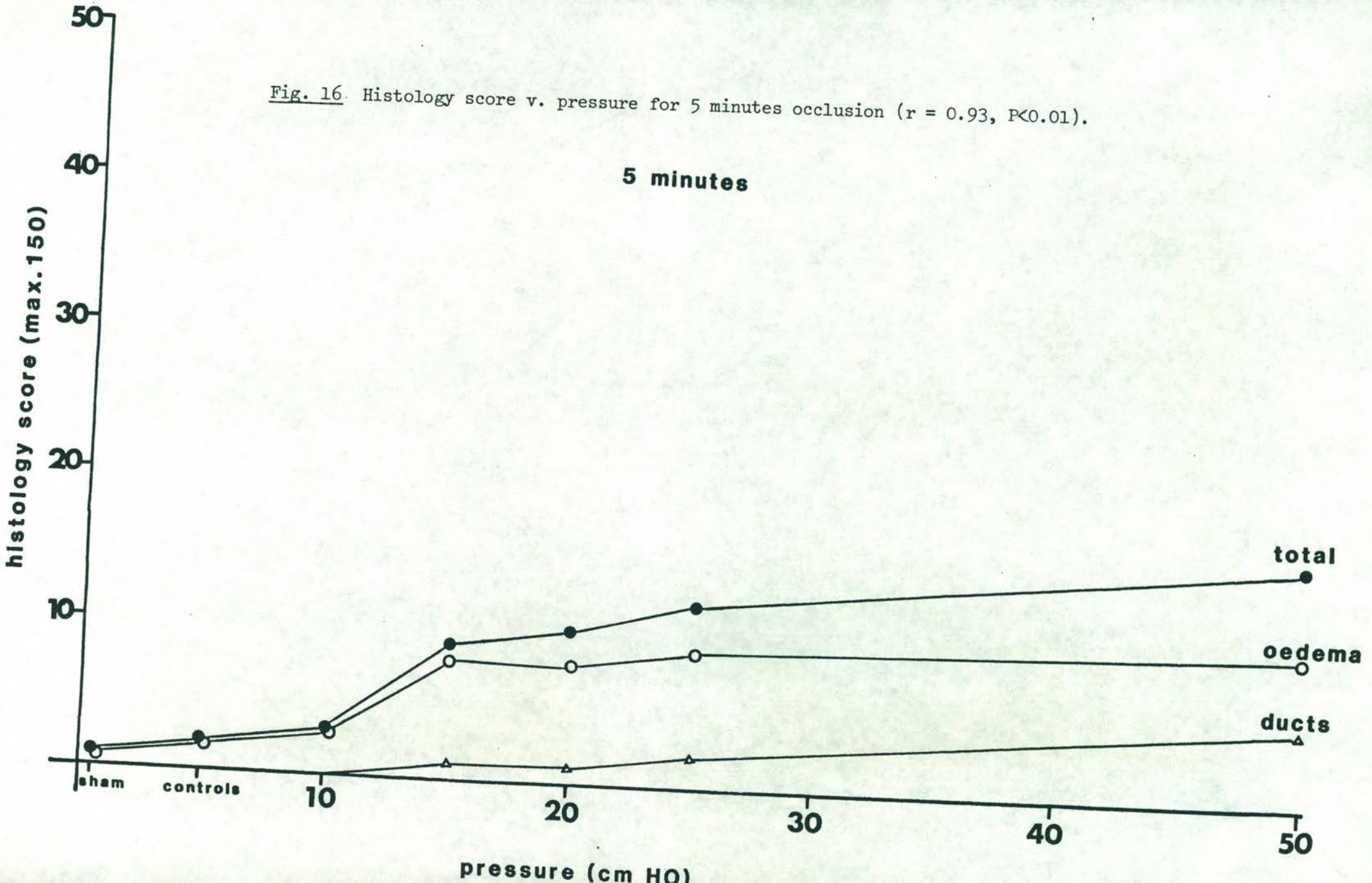


Fig. 16 Histology score v. pressure for 5 minutes occlusion ($r = 0.93$, $P<0.01$).

Fig. 16 Histology score v. pressure for 5 minutes occlusion (r = 0.93, P<0.01).

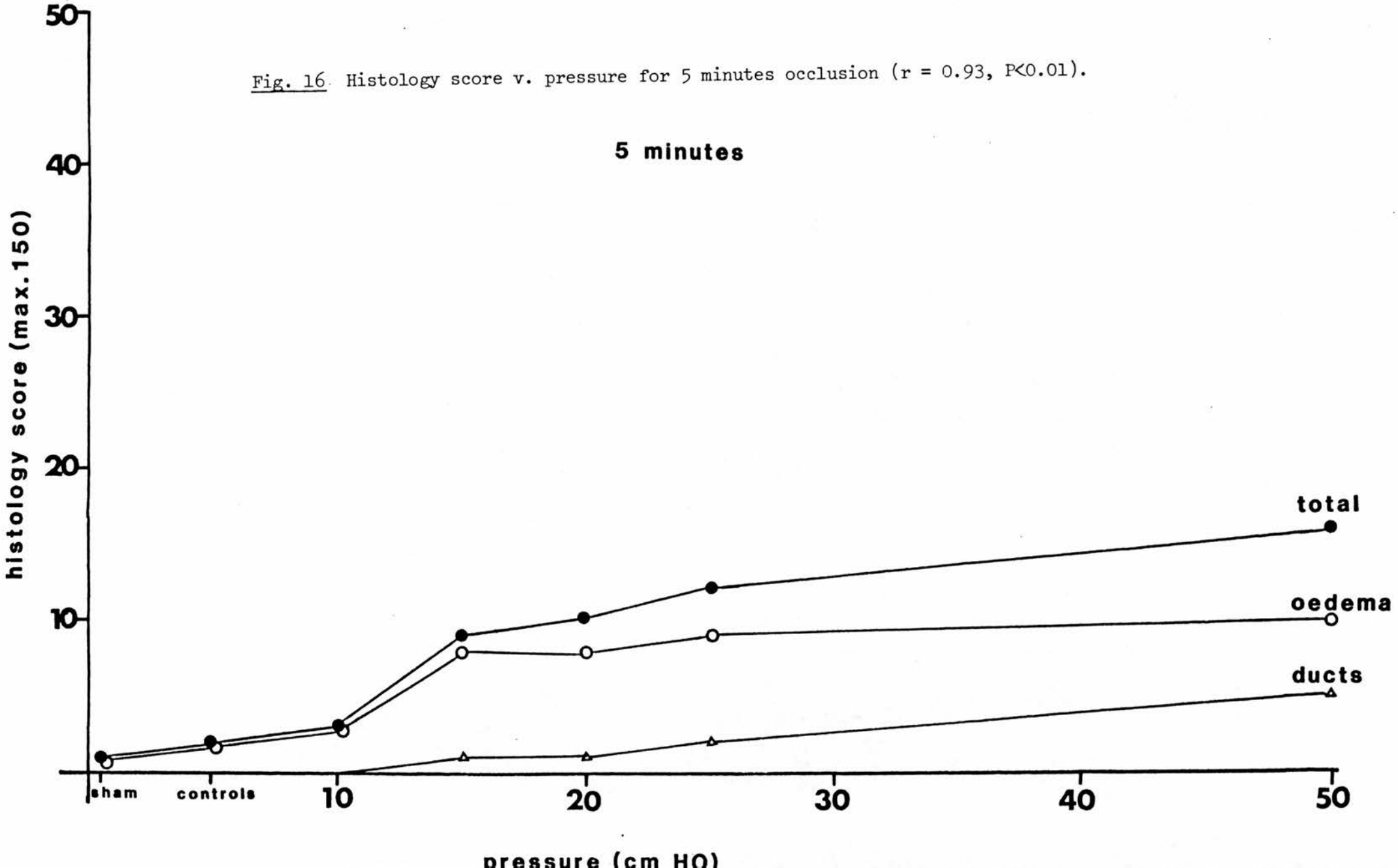
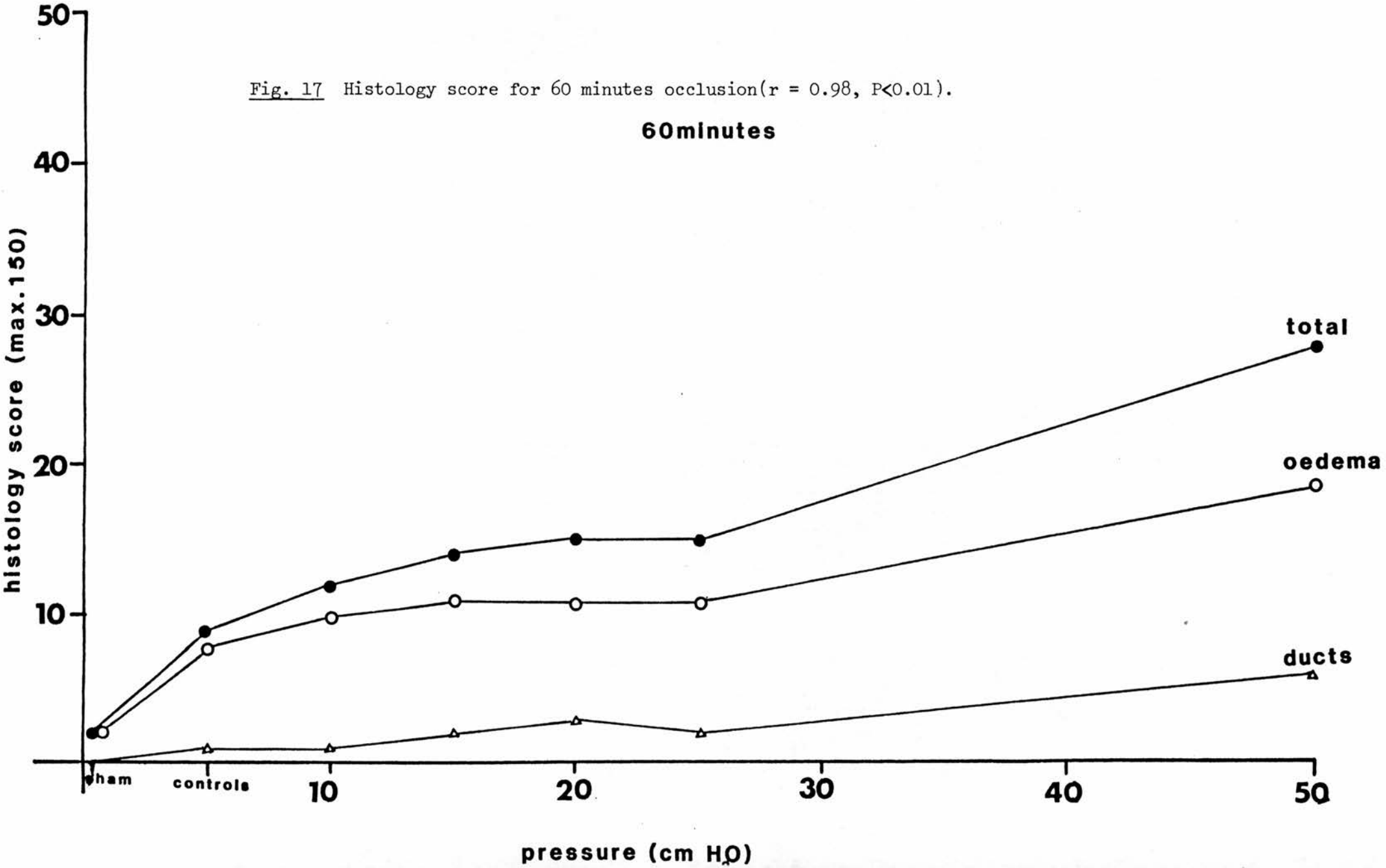


Fig. 17 Histology score for 60 minutes occlusion($r = 0.98$, $P<0.01$).



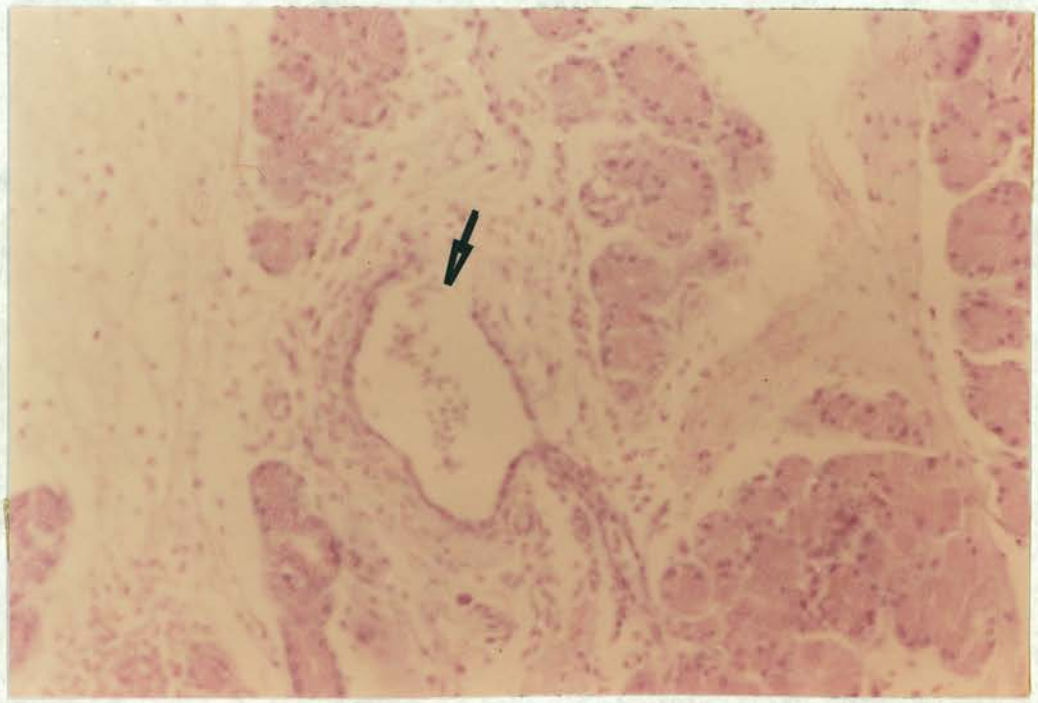


Fig. 18 Duct rupture (arrowed) (x 200).

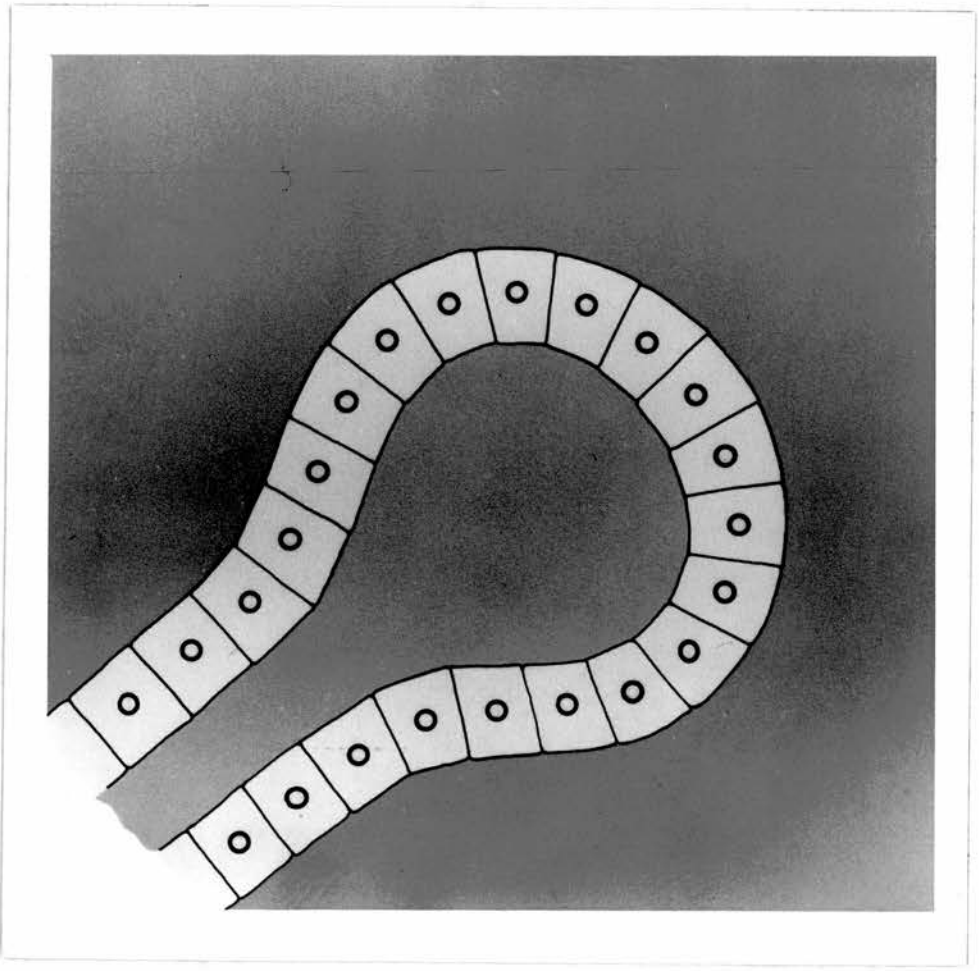


Fig. 19A Normal duct and acinus.

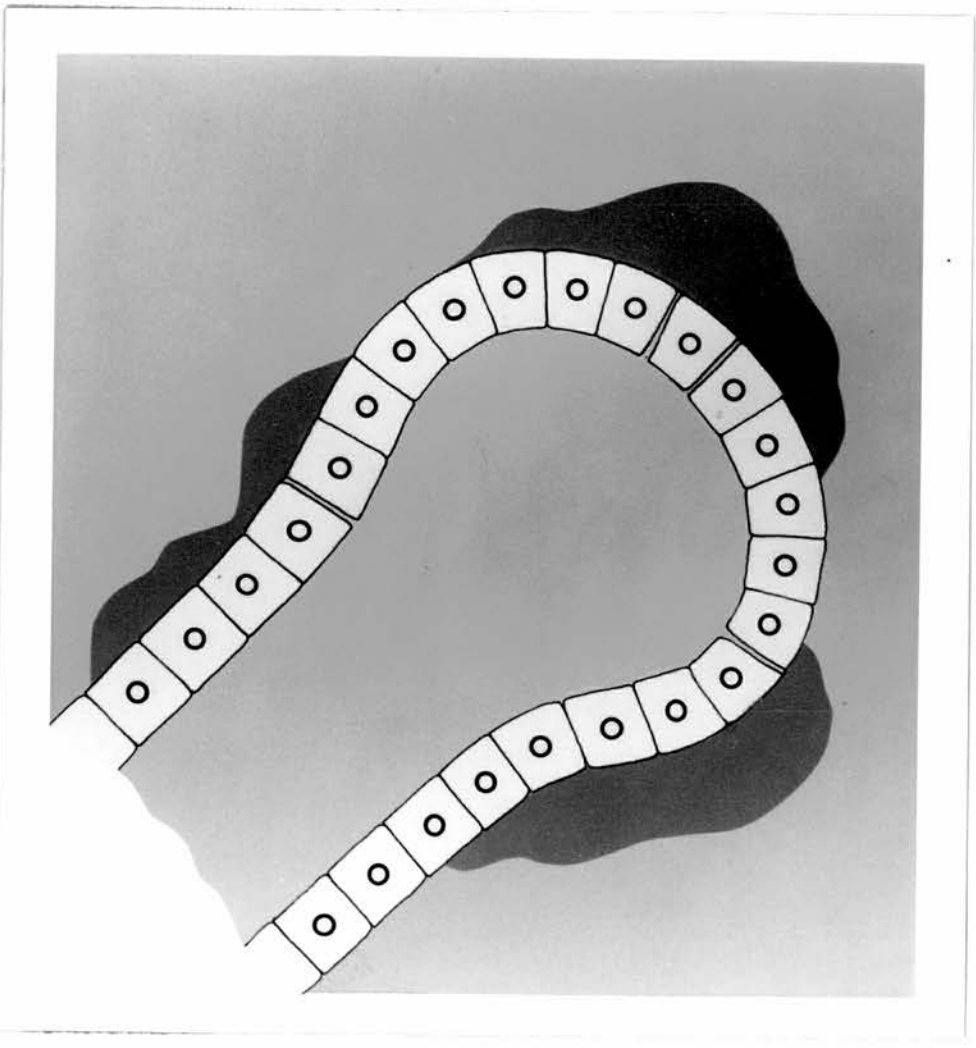


Fig. 19B Distended duct with extravasation after pressure applied.

Discussion

This experimental work has emphasized the importance of pressure in producing pancreatic damage. The preparation described gave reproducible results at each of the pressures investigated. As discussed in the previous chapter a volume of 50 µl was used in all studies to equate with that volume likely to reflux in man. Measurement of pressure within our system was difficult because of the small size of the cannulae but with practice gave good results. We confirmed the accuracy of the pressure measurements by comparison between two completely different transducer system. The transducer/recorder method of measuring pressure has many advantages over the manometric methods, not least the rapid response of the system to small changes in pressure. When injecting a constant volume into a closed system there will be variation in the time of injection at the varying pressures with the higher pressures requiring a shorter period of infusion. Thus at 10 cm H_2O the infusion required $5\frac{1}{2}$ minutes and at 50 cm H_2O, 3 seconds. Following disconnection of the pump there was a steady fall in the intraduct pressure presumably as a result of redistribution of duct contents and escape of duct contents into the interstitium. The pressures evaluated in this study were chosen as they are those likely to occur in the physiological situation. The maximum pressure of 50 cm H_2O may well occur, as mentioned in the introduction, during retching and coughing. We deliberately did not evaluate the grossly elevated pressures of 100 cm H_2O and above.

An initial study on a few rats gave fasting biliary (8 cm H_2O) and pancreatic (11 cm H_2O) pressures in close agreement with previous observations (Menguy 1958, Parry 1955). Whereas pancreatic pressure

was above biliary pressure most of the time (82%) there were episodes of pressure gradient reversal (18% of the time). When a common channel was created bile flowed into the pancreas after an initial period of stability confirming the reports of Gamklou (1966), Herriott (1966) and Hansson (1967). Thus, in the rat as in the dog (Hansson, 1967), obstruction of the common channel can produce a reversal of the normal pancreatico-biliary pressure gradient. i.e. the pancreas is more sensitive to obstruction than the biliary secretory mechanism.

In these experiments the animals were allowed to recover with free access to food and water. The results obtained therefore reflected the effect of short term pressure on the pancreas in the relatively long term setting. We investigated two periods of occlusion - short (5 mins) and long (60 mins) - as temporary occlusion of the ampulla in man by a gallstone may well be of such a time scale (Acosta 1974). Occlusion for 24 hours was not studied as the purpose of the experiment (i.e. pressure effects) becomes lost in the obstructive changes produced in the pancreas (Block 1955, Gamklou 1966).

Measurement of pancreatic gland weight ratios (PGWR) correlated closely with the water content of the gland ($P < 0.001$), the total histology score ($P < 0.001$) and histological oedema. The values obtained in the control animals (sham and control) were similar to those previously reported (Richards 1964, Baba 1983a,b) confirming the validity of our measurements. Increases in infusion pressure produced a corresponding increase in PGWR with maximum values obtained at 50 cm H_2O and with a long occlusion time. The results obtained by estimating the water content of the gland (i.e. a direct measurement of the degree of oedema)

were similar. In the control animal the water content of the pancreas was constant at 68-70% (Aho 1983). This study showed a marked elevation in water content with increased pressure reaching maximum values of above 80%.

Serum amylase measurements do not reflect the degree of pancreatic damage in animals and man (Ranson 1976, Imrie 1978, Berry 1981). The production of pancreatic oedema by increased pressures has been accompanied by raised serum amylase levels in previous reports (Schiller 1974, Mallet-Guy 1958). Damage to the pancreas, therefore, will raise serum amylase levels but the degree of elevation is not proportional to the degree of pancreatic damage. Indeed transient, and usually harmless, rises in SA levels are often seen after ERCP examination (Skude 1976) and these may well be due to a pressure effect. Anderson and Schiller (1968) investigated microcirculatory dynamics in the pancreas. They postulated that enzymes pass into a periacinar space before being transported via the thoracic duct and portal vein to the systemic circulation. Damage to the pancreas appears to produce a release of amylase into the periacinar and interstitial spaces. The amylase passes into the circulation via lymphatic (Popper 1940) and vascular pathways (Howard 1949, Egdahl 1958). This study demonstrated that the serum amylase levels were extremely variable at the pressures studied with much overlap between groups. The maximum values were obtained when the pressure applied was 50 cm H_2O. Release of amylase into the peritoneal fluid appeared to be a much better marker of pancreatic damage. Amylase enters the peritoneal fluid by passive diffusion from the pancreas and is normally not detectable. In the present study peritoneal fluid amylase levels rose steadily with

increases in pressure and were maximal at 50 cm H_2O.

Histological assessment of the pancreas is the most useful way of assessing pancreatic damage; it is the "gold standard" against which other tests are compared. The histological results obtained in this study were reproducible between two independent observers, an important factor in eliminating observer bias. The changes observed in the pancreas were essentially those of oedema, possibly as a result of the known extravasation from the duct system. Indeed Herriott (1966) in his study with Indian ink noted that extravasated ink was associated with PAS (periodic acid Schiff) positive material. This positive material appeared to be of intraductal origin supporting the concept of duct leakage. In this study low pressures ($<$15 cm H_2O) produced no evidence of duct leakage and the pancreatic damage was minimal. At moderate pressures (20, 25 cm H_2O) histological evidence of oedema increased in keeping with the known leakage from intercellular clefts. At high pressure (50 cm H_2O), when duct rupture was common, there was evidence of gross oedema in the pancreas. In addition evidence of duct dilatation and escape of ductal contents with associated inflammation became apparent. At no pressure or occlusion time, however, did the histology score for one gland exceed 4 i.e. mild pancreatic damage only. There was no evidence of acute pancreatitis.

The pancreatic ducts are a relatively low pressure system. When the pressure in the ductal system exceeds 20 cm H_2O there is marked leakage from the lumen through intercellular clefts confirming the observations of Pirola (1970) and Gambill (1973). The studies of Anderson and colleagues (1968) suggest that this leakage is a physiological phenomenon,

i.e. a method of control of intraluminal pressure. The leakage of normal duct contents produces interstitial oedema with later escape into the vascular and lymphatic systems. At higher pressures still, unphysiological duct rupture occurs with associated oedema. Pressure in the duct system is important in the context of extravasation of duct contents. If the duct contents are of a more toxic nature than the saline used in these experiments it appears likely that acute inflammation will result. Indeed as some toxic agents affect the permeability of the duct wall (Konok 1969, Reber 1979) leakage from the ducts might occur at lower pressures than those described, thus giving more credibility to the theory of duct leakage.

Finally it is our belief that careful control of pressure is an essential prelude to a physiological study of acute gallstone pancreatitis.

Conclusions

1. Increasing pressure in the pancreatic ducts will produce duct extravasation with oedema and elevated enzyme levels.

2. Leakage through intercellular clefts occurs at pressure of 20-25 cm H_2O, and ducts rupture at higher pressures.

3. There is an important relationship between the pressures in the biliary and pancreatic ducts.

4. Control of injection pressure (and volume) is an essential prerequisite of meaningful studies on the aetiology of acute gallstone pancreatitis.

CHAPTER V

BILE, INFECTION AND THE PANCREAS

Introduction

Interest in the role of bile in acute gallstone pancreatitis has remained high since the initial observations of Opie in 1901. Opie's first article (1901a) noted at autopsy a stone in the distal common bile duct of a patient who had died of severe pancreatitis associated with a pancreatic abscess. He reasoned that the stone had at one time produced temporary obstruction of the duct of Wirsung causing pancreatitis. In the context of the concept of migration of stones causing pancreatitis (Acosta 1974, Kelly 1976), it is more likely that the gallstone that was responsible for the fatal pancreatitis had already been passed into the duodenum, and that stones retained within the common bile duct precipitated additional episodes of pancreatitis. His second publication (Opie 1901b) reported a case of fatal haemorrhagic pancreatitis associated with a stone that was impacted in the ampulla of Vater. He reasoned that the impacted stone enabled bile to reflux from the common bile duct into the pancreatic duct causing pancreatitis. It is more likely that the temporary impaction of a gallstone in the ampullary region causes regurgitation of bile into the pancreatic duct and a permanent impaction as demonstrated by Opie occurs only rarely.

Opie's theory fell into disfavour for many years and it was not until the important observations of Acosta (1974) and Kelly (1976) that interest was rekindled in the role of bile in the pathogenesis of acute gallstone pancreatitis.

Bile (Hermann 1979)

The amount of bile produced each day by man is estimated to vary from 500 to over 1200 ml, depending upon the method of collection, the amount and type of diet, the state of hydration, and the influence of other stimuli such as vagal stimulation, secretin, and the reabsorption of acids. Normal hepatic bile contains about 97% water with an alkaline pH of between 6.0 and 8.5; and a specific gravity of approximately 1.040. The gallbladder concentrates bile six to ten fold by water absorption and secretes mucin into the bile (approximately 20 ml/day). The bile constituents are:- bile salts/glycine and taurine conjugates, phospholipids (lecithin), cholesterol, mucin, bilirubin/glucuronic acid, lipids, electrolytes, vitamins and enzymes.

Pancreatic juice (Hermann 1979)

The amount of pancreatic juice produced daily by man varies from 700 to 2000 ml, depending upon the state of hydration, the health of the patient, and stimuli to secrete pancreatic juice. Pancreatic juice is a clear, watery secretion that is alkaline in pH (8.0 to 8.7) with its consistency varying from a thin, watery fluid to a thicker, more viscid fluid. The specific gravity ranges from 1.007 to 1.042. The pancreatic juice constituents are:- electrolytes, glucose, protein, and enzymes including amylase, trypsinogen, chymotrypsinogen, carboxypeptidases A & B, leucine aminopeptidase, trypsin inhibitor, lipase, ribonuclease, deoxyribonuclease, elastase, collagenase and lecithinase (Harper 1972).

Bile and the pancreas

The role of bile in the initiation of acute gallstone pancreatitis is contentious. A recent pathological study by Foulis (1980) has

demonstrated that the initial pancreatic damage observed in acute gallstone pancreatitis is that of primary duct inflammation, with subsequent inflammation and necrosis of the pancreatic parenchyma surrounding the excretory ducts. This observation suggests that gallstone pancreatitis is principally resultant on duct damage following bile or duodenal reflux into the pancreatic duct system.

A critical review of the literature on the role of bile in pancreatitis was written by Grossman in 1959. He was led to the conclusion that pancreatitis, resultant on bile injection into the pancreatic ducts, would only occur when the pressures used were great enough to cause mechanical rupture of the acini. Indeed bile does frequently reflux into the pancreatic duct without inducing pancreatitis (Taylor 1981, Mosley 1981). Therefore, even if bile does reflux from the common bile duct into the pancreatic duct under normal conditions, its composition must be altered amongst patients with nonobstructive biliary disease to cause pancreatitis on the basis of physiological reflux (Banks 1971).

Elliott and co-workers (1957, 1971) demonstrated that bile incubated with pancreatic juice or trypsin was much more damaging to the pancreas than bile alone. They postulated that the proteolytic enzymes of pancreatic juice degraded a substance in bile (possibly mucoprotein) which modified the ability of bile to penetrate and injure the pancreas. This theory of mixing of bile and pancreatic juice had initially been proposed by Popper (1948). He found that 83% of patients had very high amylase levels in gallbladder bile early in acute pancreatitis, whereas high amylase was found in only 10% at elective cholecystectomy. Recent studies by Nevalainen (1980) have suggested that this toxic mixture might be as a

result of pancreatic phospholipase A_2 acting on biliary lecithin to produce lysolecithin.

The information resultant on numerous studies of bile toxicity on the pancreas suggests that:

(i) Normal bile at low pressures produces little pancreatic damage and probably never acute haemorrhagic pancreatitis when injected into the pancreas

(ii) bile may reflux into the pancreatic duct without initiating acute pancreatitis

(iii) acute pancreatitis at physiological pressures can only be produced by mixing bile with other substances and then infusing the mixture into the pancreas.

These observations therefore suggest that normal bile alone is insufficient to cause pancreatic inflammation.

Bile and pressure

The toxic potential of bile following reflux from the common bile duct into the pancreatic duct remains uncertain (Banks 1971). Experimentally, bile produces significant pancreatic inflammation when refluxed at high pressures (Sum 1970, Rittenbury 1969, Gilsdorf 1967) but not at more physiological pressures (Robinson 1963, White 1960).

The experiments of Robinson and Dunphy (1963) have been much quoted as an example of the inability of bile to produce pancreatitis at low pressures. They used an experimental preparation in the goat by which all the bile passed through the pancreas and thence into the bowel. The

early changes produced in the pancreas were those of mild oedema, ductal dilatation and minimal inflammation. Long term changes of ductal fibrosis were apparent after several months. Even though bile passed through the duodenum, and thus gained enterokinase, there was no evidence of acute pancreatitis. This study confirmed an earlier report of White (1960) that the normal pancreas presented considerable resistance to damage by bile at low pressures. Animal experiments have therefore shown that sterile bile alone is relatively innocuous to the pancreas if the solution is delivered at a low pressure (Mann 1923, White 1960, Robinson 1963, Elmslie 1966, Keynes 1981).

When sterile bile is infused into the pancreas at elevated pressures then pancreatic inflammation does occur. Blumenberg and Powers (1963) anastomosed biliary and pancreatic catheters in dogs. They found that retching increased the bile duct pressure to values in excess of those in the pancreatic duct and that bile flowed into the pancreas. A comparison of the relationship of biliary-pancreatic ductal pressure gradient to the severity of pancreatitis showed the more severe pancreatitis to be associated with a higher biliary-pancreatic pressure gradient. Similar results were reported by Hermann and Knowles (1965) who importantly emphasized that the biliary system of dogs is almost universally contaminated with pathogenic organisms. Thus infection complicates these studies (see later).

Gilsdorf and colleagues (1967) devised an ingenious way of studying the effects of pressure and bile on the pancreas. They increased pancreatic ductal pressures by autonomic stimulation of the hypothalamus and coeliac ganglion. Pancreatic inflammation initiated by bile infusion was

invariably more severe in those animals undergoing autonomic stimulation with an attendant increase in mortality from 25 to 90%. In other experiments (Keynes 1981) the main pancreatic duct when anastomosed to the side of the common duct gave pancreatitis if the dogs were given morphine with a rise in biliary pressures.

Similar experiments have been performed in rats by ligating the distal bile-pancreatic ducts (Block 1955, Gamklou 1966, Wanke 1970). When biliary pressure was increased by the administration of CCK or feeding then acute pancreatitis developed in most animals.

Bile infused into the pancreas at high pressures causes acute pancreatitis. This may be due to bile escaping from the ducts through the previously described intercellular clefts or frank ruptures into the interstitium of the pancreas. The possibility of these pressures being generated in the physiological context has been considered in the previous chapter.

"Pancreatitic bile"

Little is known about the composition of bile in patients with acute gallstone pancreatitis ("pancreatitic"). The nature of bile is important in consideration of either the bile-pancreatic or the duodeno-pancreatic reflux theories of initiation. Indeed why should so many patients with acute pancreatitis have gallstones and yet only 5% of patients with gallstones develop pancreatitis (Schmidt 1976, Banks 1979, Braganza 1983); the passage of a stone through Oddi's sphincter would promote reflux, but reflux would cause pancreatitis only if the bile contained excessive amounts or abnormal types of toxic substances.

Hansson (1963, 1967) investigated free bile acids in acute pancreatitis. He found free bile acids to be almost twice as toxic for the pancreas as their conjugates and thus postulated that the occurrence of free bile acids (normally present in insignificant amounts in bile) in the bile might be of significance in the pathogenesis of acute pancreatitis. He found free (unconjugated) bile acids in the bile of 10 of 45 patients with acute pancreatitis and 9 of 425 patients with gallstones only. Further study could find little difference in the dihydroxy : trihydroxy ratio of the bile salts. Hansson concluded that "free bile acids did not infrequently occur in the bile in patients with acute pancreatitis." Furthermore, since free bile acids were more toxic than their conjugates, bile containing free bile acids would probably produce more severe damage to the pancreas than bile of normal bile acid composition.

Banks (1971) considered that in health bile refluxed from time to time from the common bile duct into the pancreatic duct without resultant damage. When this bile contained "toxic" substances then pancreatic damage might supervene.

Recent experiments by Sanfey (1983), Parks (1983) and Braganza (1983a,b) have postulated that aberrant function of the hepatic mixed function oxidases is the root cause of pancreatic disease. This aberrant function is associated with biliary excretion of lipid peroxidation products, toxic epoxides, carcinogens and free radicals. Factors that promote reflux into the pancreatic duct would increase the risk of pancreatic disease only when the bile contains excessive amounts or abnormal types of reactive intermediates.

A review of the literature on the subject of "pancreatitic" bile reveals major deficiencies in our knowledge and that suggests that further study of such bile is warranted.

Infection, bile and the pancreas

In the early part of this century, infection was widely accepted as a cause of acute pancreatitis, and Fitz (1889) who first described acute haemorrhagic pancreatitis in man believed that acute pancreatitis originated by extension of a gastroduodenal inflammation along the pancreatic duct. There has been a lack of bacterial data in clinical acute pancreatitis, on the assumption that the disease is a purely chemical digestion of the gland. Hermann (1979) has suggested that gallstones might cause pancreatitis by introducing bacterial infection into the bile-pancreatic juice mixture. This could change the physiological characteristics and chemical properties of both bile and pancreatic juice and thus permit the flow of the incubated mixture into the pancreatic duct system more readily under lower pressures.

There has been much study of the bacterial content of bile in patients with various biliary tract and pancreatic diseases (Keighley 1978, Lennette 1980, Chetlin 1971, Cox 1978, Delias 1977, Goswitz 1974, Lou 1977, Dye 1978, Lötveil 1978). Although various routes have been suggested for the transport of micro-organisms into the bile, the exact mechanism by which infection occurs is not yet clearly elucidated (Scott 1967, 1971, Dineen 1964). It seems likely that infection of the bile by transport of bacteria from the duodenum is the most plausible hypothesis (Suzuki 1984, Jackman 1980), although the possibility of haematogenous (Shou 1968, Scott 1967) or lymphogenous (Graham 1922,

Byrne 1960) transport of bacteria has also been emphasized. Patients with gallstones have infected bile in up to 50% of cases (Taylor 1981, Keighley 1978). If the stones are in the gallbladder only then there is at least a 30% biliary infection rate. When these stones are present in the common bile duct then the infection rate rises to 70% (Keighley 1982). Accepting the gallstone migration theory, suggests that the bile of patients with gallstone pancreatitis may contain bacteria in a significant proportion of cases. A recent careful study by Suzuki and colleagues (1984) analysed transhepatic aspirated bile and produced results more accurate than bile taken at operation. They found the overall incidence of biliary infection in patients with bile duct stones to be 90% and even higher (95%) in patients with a dilated bile duct and ampullary stones. The most frequent bacteria isolated were E. coli, Klebsiella, enterobacteria, proteus and mixed infection was common. Moreover, simultaneously collected duodenal fluid showed a 86% coincidence with intrahepatic bile in terms of positive or negative cultures. These studies have demonstrated that biliary ~~bacteraemia~~ infection is common in patients with gallstones and almost universal when there is choledocholithiasis.

Thal and colleagues (1956) infused various suspensions of bacteria into the pancreatic duct at pressures below 40 cm H_2O. Acute haemorrhagic pancreatitis occurred only with bacteria that gave an intradermal necrotizing lesion with small vessel occlusion i.e., coliforms, clostridia, and staphylococci. The conclusion was that the pancreatitis and intradermal necrosis resulted either from enzymes from the bacteria or their toxins (Keynes 1980). Earlier experimental work by Flexner (1901) had shown that intraductal infection of E. coli and pseudomonas gave pancreatitis. E. coli itself has been shown to have mucolytic and cytotoxic effects on

pancreatic ductal cells (Keynes 1981) and the bacterial production of haemorrhagic pancreatitis appears usually to require infection with particular bacteria capable of producing cytotoxins.

Acute gallstone pancreatitis has previously been associated with acute cholecystitis. Acute gallbladder and acute pancreatic inflammation appear to coexist in 10-20% of cases (Ivy 1952, Blumenthal 1959). It has been further shown that there are lymphatic pathways between the gallbladder and pancreas (Weiner 1970, Gambill 1973) and that bacteria could travel along this route. However, despite these observations bacteria appear to have their most significant effect on the pancreas through their occurrence in bile.

Poncelet and Thompson (1973) studied the effect of infected bile on the feline sphincter of Oddi. The normal resting sphincteric resistance was noted to be 14 cm H_2O and this was unaltered by either normal bile or conjugated bile salts. In contrast infected bile produced a 115% increase (to 30 cm H_2O) in sphincteric resistance and this was postulated to be due to the production of unconjugated deoxycholic acid by bacterial action.

Pancreatic abscess is a rare but extremely serious complication of gallstone pancreatitis. A review of the organisms responsible for this infection shows that E. coli, Klebsiella, proteus and enterococci are the most frequently found (Frey 1979, Camer 1975, Holden 1976, Kodesch 1973). Whether these abscesses develop as a result of primary infection or secondary superinfection remains debateable.

Further studies on the role of bacteria in experimental acute

pancreatitis have been performed using the duodenal loop preparations (Pfeffer 1959, Strack 1967, Williams 1968, Ferrie 1978, Keynes 1980, Byrne 1964). Chetty and colleagues (1980) described a new model for the production of acute haemorrhagic pancreatitis. They injected infected bile into a closed loop and produced consistent severe pancreatic damage indicating that a combination of pressure, bile reflux and infection was markedly toxic to the pancreas. When antibiotics are added to the duodenal loop, with subsequent control of the bacterial component, there is a marked decrease in the incidence of haemorrhagic pancreatitis in dogs using the closed loop technique (Keynes 1980). Keynes concluded that experimental interstitial pancreatitis resulted from damage to the pancreatic duct system without infection, and haemorrhagic pancreatitis occurred after reflux of bacteria into the pancreatic ducts from the duodenum. Only bacteria such as E. coli and Clostridia that produce proteolytic enzymes and cytotoxins appeared able to cause haemorrhagic pancreatitis. He further demonstrated that in haemorrhagic pancreatitis such bacteria were found in the pancreas, but none were identified in interstitial pancreatitis. The author endorses his conclusion that there is a great need for clinicians to consider the bacteriology of acute pancreatitis more closely with duodenal aspirations or endoscopy with pancreatic duct intubation.

Konok and Thomspon (1969) investigated the effect of bile and infection on the integrity of the pancreatic ductal mucosa. They suggested that the pancreatic ductal wall with its covering layer of mucus serves as a protective barrier between the ductal contents and the pancreatic parenchyma with a defect in this barrier being the earliest step in the evolution of AGP. This idea formed the basis of the concept of a pancreatic duct

mucosal barrier as described by Reber and colleagues (Reber 1979). (see Chapters 8-12). Konok and Thompson (1969) found that the pancreatic ducts were impermeable to normal bile and suspensions of E. Coli at low pressures whereas they were considerably damaged by bile infected with E. Coli. The infected bile appeared to show mucolytic and cytotoxic effects far beyond those of the other solutions tested. Reber and co-workers (1979) demonstrated that the pancreatic duct mucosal barrier was damaged by bile infected with biliary E. Coli but not by bile infected with urinary E. Coli. Foulis (1980) has further shown that a low pressure intraduct infusion of E. Coli admixed with duodenal juice produces an initial lesion of severe duct inflammation with necrosis of the duct wall.

Mizumoto and associates (1971) reported on the effect of bacterial mucopolysaccharidases (β gluconidase, hyaluronidase) on the pancreatic ducts. They hypothesised that the toxicity of infected bile might be due to beta gluconidase produced by bacteria such as E. Coli. Sterile bile and β glucuronidase given individually did not damage the pancreatic ducts. In contrast their admixture produced disappearance of the thin mucous layer and goblet cells of all the pancreatic ducts, and moreover destruction of some ducts was associated with severe necrosis and round cell infiltration of the pancreatic tissue. The enzyme β-glucuronidase also converts bilirubin diglucuronide to free bilirubin (Banks 1971, Small 1968) but it appears unlikely that bilirubin itself is toxic to the pancreas (Poncelet 1972).

Creutzfeldt (1970) has suggested that bacterial endotoxins can interact with blood and trypsin to produce some factor which can cause haemorrhagic

pancreatitis . This hypothesis was supported by Williams & Byrne (1968) who reported E. Coli to contribute an endotoxin which reacted with blood to produce haemorrhagic changes. It is of interest that endotoxins from bacteria are now being isolated from the blood of patients with severe acute pancreatitis (Keynes 1981).

Bacteria alter the chemical properties of bile. Infection in the biliary tree converts all phospholipids to the lysolecithin form which is known to be extremely toxic to the pancreas (Poncelet 1972) (see later). Certain bacteria are known to be capable of deconjugating bile salts e.g., bacteroides, streptococcus faecalis, clostridia (Banks 1971, Gorbach, 1969, Rosenberg 1969). Hansson (1967) extensively investigated the ability of clostridia and enterococci to hydrolyze the bile acids in bile. Clostridia hydrolyzed most of the conjugated bile acids and enterococci in one third of samples. He concluded that bacterial hydrolysis of the biliary bile acids could occur in infection of the biliary tract and this might have relevance in the context of free bile acids being twice as pancreatotoxic as the conjugated variety. Keynes (1980) reported that the toxic potential of infected duodenal fluid was much reduced after bacterial filtration through a Seitz filter suggesting that both the bacteria themselves (endotoxins) and their exotoxins might be important in producing pancreatic damage.

These observations on the role of infection and bile can be summarized as

(i) infected bile under the same physiological conditions is much more toxic to the pancreas than sterile bile.

(ii) infected bile can produce severe pancreatic damage even at very low pressures

(iii) infected bile has cytotoxic and mucolytic properties that damage the duct wall and increase its permeability.

(iv) infection may have a role in the conversion of interstitial oedematous pancreatitis to the haemorrhagic form.

(v) bile and infection individually are much less toxic than after incubation together, which suggests that infection induced some change in the bile.

(vi) infection may increase the toxicity of bile by deconjugating bile salts, converting lecithin to lysolecithin, or by producing exo and endotoxins.

Although the role of bile in the initiation of acute gallstone pancreatitis has been the source of much debate there appear several questions as yet unanswered. For instance, what is the physiological role of bile at normal volumes and pressures in producing pancreatic ductal damage? Do patients with acute pancreatitis have an altered chemistry of bile? What role, if any, does infection have in the initiation of gallstone pancreatitis? A summary of all the previously mentioned observations appears to indicate

I. Sterile bile does not cause acute pancreatitis at physiological pressures.

II. The chemical nature of bile in pancreatic disease remains unknown.

III. Infected bile is extremely toxic to the pancreas.

These observations and the many unanswered questions prompted a study of the effect of bile and infection on the pancreas. Further investigation of their effects on the pancreatic duct mucosal barrier is given in Chapter VIII.

Materials and Methods

Object

To investigate the role of the varying types of "bile" and infection in the pathogenesis of acute gallstone pancreatitis. The effects of pressure and volume, alluded to in the two previous chapters were carefully considered. This study attempted to produce standardized lesions by a careful consideration of Elliott's postulates (1971) and the following factors were precisely controlled.

(i) pressure

(ii) volume and solution introduced

(iii) time during which it is applied

Experimental preparation

The experimental preparation described in figure 5 was used throughout this study. A volume of 50 µl infusate was infused into the pancreas at pressures of 15, 20, 25 and 50 cm H_2O with cannula occlusion times of 5 or 60 minutes. The animals made a full recovery with sacrifice at 24 hours and assessment of pancreatic damage as previously described. In this study a number of animals died within the 24 hour period and these animals all underwent post-mortem examination within an hour of death.

Infusates (No patient from whom bile was removed was receiving drug therapy).

E. Coli solution

This solution was prepared by the department of bacteriology. An Escherichia Coli suspension (from biliary organisms) was freshly prepared 1 hour before use with normal saline to a strength of 10^5 - 10^6 organism/ml. Culture of the suspension confirmed the viability of the organisms.

Sterile bile ("standard" bile) (mean pH = 7.66, range 7.5-7.8)
Choledochal bile was taken at operation from 10 patients undergoing elective cholecystectomy. None had had either a previous attack of pancreatitis or were receiving antibiotics. The bile was stored and used within 24 hours in all cases. Immediately before infusion the bile was cultured and if positive the results were discarded. Thus all the bile was sterile for aerobic and anaerobic organisms as well as fungi and yeasts. Each specimen of bile gave similar results.

"Pancreatitic" bile: (mean pH = 7.12, range 6.95-7.4)
Choledochal bile was taken from 4 patients undergoing cholecytectomy for a recent (10-20 days previously) attack of acute gallstone pancreatitis. In every case the pancreatitis appeared to have settled before operation and no patient had received antibiotics before surgery. The bile did not contain active trypsin or amylase and cultures of bile were negative in each patients. The bile was infused within 12 hours of collection and each "pancreatitic" bile specimen gave similar results.

Infected bile: (mean pH = 7.53, range 7.3-7.7)
Choledochal bile was taken from eight patients undergoing elective cholecystectomy and exploration of the common bile duct for biliary lithiasis. No patients had received antibiotics before the bile specimen was taken. Culture of the bile gave growth of organisms; (No anaerobes found), E. Coli only (4), E. Coli + proteus species (2), E. Coli + Klebsiella ozonae (1), E. Coli + Streptococcus faecalis (1). Each specimen was infused into the experimental preparation within

12 hours of collection. There appeared to be little difference between the effects produced by the 8 samples.

Bile from two additional patients contained pseudomonas pyocyanea with E. Coli (1) or proteus mirabilis (1) organisms and was considered separately.

Filtered bile:

Portions of infected bile from the eight patients mentioned were passed through a bacterial filter (4.5 microns). All filtered bile specimens were negative for bacterial growth and the size of the filter enabled bacterial toxins (exo and endo-toxins to pass through. The filtered bile was infused into the experimental preparation within 30 minutes of filtration. All samples gave equivalent results.

Results (summarized in tables 7-10)

(1) E. Coli solution:

The results obtained from infusion of E. Coli solution into the rat pancreas were very similar to those obtained using saline only. At corresponding pressures and occlusion times PGWR (fig. 20A), serum (fig. 20B) and peritoneal fluid (fig. 20C) amylase levels were indistinguishable between sterile and infected saline. The histology score (fig. 20D) was greater with the E. Coli infusion when a pressure of 50 cm H_2O was employed ($P<0.02$). The microscopic changes were those of oedema and increased inflammatory cell infiltrate, no doubt as a result of escape of organisms into the intestinal fluid from the duct ruptures produced at this high pressure. Again there was no evidence of worse than mild pancreatic damage. No animal died and no animal developed acute haemorrhagic pancreatitis.

(2) Sterile bile:

All specimens gave very similar results. Macroscopically the pancreas was enlarged with little inflammation but no haemorrhage. A few fat necroses and a small amount of peritoneal fluid were present. At all pressures the PGWR was significantly higher than after saline infusion ($P<0.001$) (fig. 20A) and at 50 cm H_2O of pressure the PGWR was 35% above that of saline alone. Likewise there were significant elevations in serum ($P<0.02$) (fig. 20B) and peritoneal fluid ($P<0.01$) (fig. 20C) amylase levels. The histological changes of mild to moderate pancreatic damage were significantly greater than after saline alone ($P<0.02$)

(fig. 20D). There was microscopic evidence (fig. 21A) of acute inflammatory infiltrate with small areas of acinar necrosis and mild duct inflammation. The changes seen were most noticeable for the long occlusion time and at the higher pressures. At pressures known to be associated with extravasation through intercellular clefts there was only evidence of mild to moderate pancreatic damage. The maximum damage occurred at the high pressure with known duct rupture.

No animal died and only one (50 cm H_2O pressure) developed acute haemorrhagic pancreatitis.

"Pancreatitic" bile

The pH of "pancreatitic" bile (7.66; 7.5-7.8) was significantly lower than sterile (7.66; 7.5-7.8, $P < 0.001$) or infected bile (7.53; 7.3-7.7, $P < 0.01$).

The macroscopic appearances of the pancreas were consistent with more severe pancreatic damage than that seen after sterile bile infusion. There was more inflammation, several animals had evidence of haemorrhage, a higher volume of peritoneal fluid and larger areas of fat necrosis. The PGWR (fig 20A) was higher at corresponding pressures and occlusion times than that of sterile bile although this difference only reached statistical significance at pressures of 20 and 25 cm H_2O ($P < 0.01$). Likewise serum amylase levels tended to be higher (fig. 20B). Peritoneal fluid amylase levels were significantly higher than those after infusion of sterile bile at pressures of 25 and 50 cm H_2O ($P < 0.02$) (fig. 20C).

The histological appearances at all pressures were significantly different ($P < 0.01$). The histology scores (fig. 20D) obtained revealed moderate to severe damage at 50 cm H_2O. These changes were much more severe than those seen after sterile bile infusion. Microscopically there was evidence of gross oedema, inflammatory infiltrate, duct damage, acinar necrosis and several glands showed marked haemorrhage (fig. 21B). The mortality and haemorrhagic pancreatitis rates are shown in table 11. Six animals developed acute haemorrhagic pancreatitis of whom five died, further evidence of the increased pathogenicity of "pancreatitic" bile. All samples of "pancreatitic" bile were similar in their effects.

Infected bile:

There was no difference between the effects produced by the various coliform organisms and those with one or two bacterial species isolated (except pseudomonas). Most glands examined showed macroscopic evidence of acute haemorrhagic pancreatitis with grossly stained ascites and fat necroses. The entire gland (head and tail) was similarly affected. The PGWR was significantly higher at all pressures than any other specimen of bile, sterile or "pancreatitic" ($P < 0.001$) (fig. 20A). At a pressure of 25 cm H_2O the PGWR was 85% greater than E. Coli solution, 66% greater than sterile bile and 48% greater than "pancreatitic" bile. Both serum ($P < 0.02$) (fig. 20B) and peritoneal fluid ($P < 0.01$) (fig. 20C) amylase levels were significantly higher than those with other "bile" samples. The histological damage resultant on infected bile infusion was much worse than

with other bile specimens. The histological score demonstrated severe pancreatic damage at all pressures, results significantly higher ($P<0.01$) (fig. 20D) than with other infusates. Microscopic evidence of oedema, ductal destruction, marked acinar necrosis and inflammatory infiltrate in addition to haemorrhage from damaged vessels was evident (fig. 21B). Examples of duct damage are give in figures 22A-E. The mortality and haemorrhagic pancreatitis rates at the varying pressures are demonstrated in table 11. 23/40 animals developed acute haemorrhagic pancreatitis of whom 21 died.

In the two specimens where pseudomonas was present in the infected bile the pancreatic damage was remarkable in several respects. All animals died within 16 hours and all had fulminating acute haemorrhagic pancreatitis with haemorrhagic ascites. Microscopically the most marked feature was vascular destruction with haemorrhage throughout the gland.

Filtered bile:

All viable organisms were removed from the infected bile by this process. However, the toxins produced and the secondary effects on bile (e.g. deconjugation of bile salts) were not affected. The macroscopic appearances were those of moderate inflammation with a few fat necroses but very little evidence of haemorrhage (i.e. much less severe than with infected bile). PGWR's were significantly ($P<0.001$) lower at all pressures than infected bile although the values were still higher than

sterile bile ($P<0.02$) (fig. 23A). Both serum ($P<0.02$) (fig. 23B) and peritoneal fluid ($P<0.01$) (fig. 23C) amylase levels were reduced compared with infected bile to values close to those obtained with sterile bile. Filtration of infected bile significantly ($P<0.01$) (fig. 20D) reduced the histological damage, although again the results were more marked than those of sterile bile ($P<0.01$). Compared with infected bile there was less marked haemorrhage, acinar necrosis and acute inflammation. 7/40 animals developed haemorrhagic pancreatitis of whom five died (table 11).

Thus, whilst filtration of infected bile significantly reduced its pathogenicity, the pancreatic damage observed was still much greater than of sterile bile. These results suggest that the organisms themselves either release toxins into the bile or alter the chemical composition of the bile itself.

Results Summary

1. E. Coli solution produced pancreatic damage indistinguishable from that after ordinary saline infusion.
2. Sterile bile at physiological volumes and pressures gave mild to moderate pancreatitis.
3. "Pancreatitic" bile was significantly more toxic to the pancreas than sterile bile.
4. Infected bile was markedly pancreatotoxic and produced a high incidence of fatal acute haemorrhagic damage to the pancreas.
5. Filtration of infected bile reduced its pathogenicity. As the damage observed was greater than that of sterile bile there

is a possible role for bacterial toxins or changes in the bile itself.

TABLE 7 Pancreatic gland weight/body weight (g/100g) (groups of 10 animals)

5 mins infusion
60 mins infusion

	SHAM	CONTROL	10	15	20	25	50
Saline	316±44 *319±68*	329±25 *344±11*	332±15 *318±19*	324±30 *394±42*	341±35 *398±23*	361±26 *396±31*	408±24 *440±48*
E. Coli				326±40 *385±40*	350±24 *395±41*	372±21 *402±36*	404±18 *436±35*
Sterile Bile				394±21.2* *412±14.1*	375±45.5* *418±14.9*	404.8±23* *430±9.3*	557±36* *591±23*
Pancreatitic Bile				417±26.1 *419±16.2*	469±30.0* *460±16.4*	470±26.1* *472±30.2*	563±38* *586±31*
Infected Bile				480±30* *496±21*	630±15.0* *604±16*	701±16* *695±17*	710±51* *750±40*
Filtered Bile				410±24.0*+ *450±16.0*	499±30.0*+ *448±17.0*	469±31*+ *485±32*	640±41*+ *631±36*

TABLE 7 LEGEND

E. coli v. saline		N.S.
Sterile bile v. saline		*P<0.001
"P'ic" bile v. sterile bile		*P<0.01
Infected bile v. all		*P<0.001
Filtered bile	v. infected bile	*P<0.001
	v. sterile bile	+P<0.02

TABLE 8 Serum Amylase (u/l)
(groups of 10 animals)

5 mins infusion
60 mins infusion

PRESSURE (cm H_2O)

	SHAM	CONTROL	10	15	20	25	50
Saline	1119±467 *1360±510*	3354±1406 *5489±3810*	4635±997 *3982±2668*	7225±2705 *5091±2027*	6467±3045 *4960±1679*	6373±2555 *5489±1511*	10295 *11494±2553*
E. Coli				8500±3100 *7540±1800*	6200±2100 *6300±3600*	6500±3000 *6800±1701*	8700±2050 *10400±1800*
Sterile Bile				9345±5618 *9244±2053*	8528±3619 *9035±3558*	10130±6072* *11818±3804*	17775±10690* *19670±8550*
Pancreatitic Bile				9500±6020 *10300±1060*	10450±4020 *12400±3100*	14100±3600 *15100±1800*	18500±9500 *19800±4060*
Infected Bile				13200±6000* *14200±1600*	18000±1600* *26000±4060*	20000±4200* *24000±1600*	23000±1460* *27000±2070*
Filtered Bile				10700±4100* *12400±1700*	147000±6000* *12600±7100*	13700±4700* *16300±3100*	17050±5000* *18250±3120*

TABLE 8 LEGEND

E. Coli v. saline		N.S.
Sterile bile v. saline		*P<0.02
"P'ic" bile v. sterile bile		N.S.
Infected bile v. all		*P<0.02
Filtered bile	v. infected bile	*P<0.02
	v. sterile bile	N.S.

TABLE 9 Peritoneal fluid amylase (u/l x 10^3) < 5 mins infusion / 60 mins infusion

PRESSURE (cm H_2O)

	SHAM	CONTROL	10	15	20	25	50
Saline		4.1±1.31 *5.1±2.1*	5.25±1.26 *6.2±1.8*	8.1±2.8 *8.4±2.2*	9.4±3.2 *10.9±3.3*	9.4±3.3 *10.5±4.4*	32.4±6.2 *34.2±5.9*
E. Coli				8.6±3.0 *8.8±2.7*	8.8±2.7 *9.6±2.7*	9.2±3.1 *9.9±4.1*	30.6±5.0 *30.9±6.1*
Sterile Bile				15.4±3.0* *16.4±3.9*	16.3±4.1* *21.0±5.6*	23.8±5.1* *23.7±6.3*	49.1±12.4* *56.1±7.2*
Pancreatitic Bile				16.3±3.6 *18.2±4.0*	18.5±4.3 *20.9±5.0*	24.6±6.0* *28.2±4.7*	56.2±14.8* *65.2±16.0*
Infected Bile				34.0±6.0 * *37.2±10.2*	43.2±7.2* *45.0±8.2*	47.1±5.2* *52.6±6.3*	78.1±26.7* *89.1±32.2*
Filtered Bile				21.2±6.1*+ *28.4±4.1*	23.4±4.8*+ *29.4±7.3*	27.6±6.2*+ *36.3±7.2*	59.6±13.2*+ *65.2±8.0*

TABLE 9 LEGEND

E. Coli v. saline N.S.

Sterile bile v. saline *P<0.01

"P'ic" bile v. sterile bile *P<0.02

Infected bile v. all *P<0.01

Filtered bile
- v. infected bile *P<0.01
- v. sterile bile +P<0.02

TABLE 10 Histology Score (0-150) (groups of 10 animals) — 5 mins infusion / 60 mins infusion

PRESSURE ($cm\ H_2O$)

	SHAM	CONTROL	10	15	20	25	50
Saline	1 *2*	2 *9*	3 *12*	9 *14*	10 *15*	12 *15*	16 *28*
E. Coli				10 *16*	12 *17*	18 *21*	30* *48*
Sterile Bile				25* *32*	28* *40*	36* *45*	55+ *68*
Pancreatitic Bile				54* *63*	63* *72*	76* *84*	77* *93*
Infected Bile				96* *122*	108* *131*	120* *130*	140* *148*
Filtered Bile				61*+ *83*	63*+ *104*	96*+ *109*	121*+ *123*

TABLE 10 LEGEND

E. coli v. saline *P<0.02

Sterile bile v. saline *P<0.02, +P<0.01

"P'ic" bile v. sterile bile *P<0.01

Infected bile v. all *P<0.01

Filtered bile
- v. infected bile *P<0.01
- v. sterile bile +P<0.01

PRESSURE (cm H_2O)

	10	15	20	25	50
Saline	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
E.Coli	—	0 (0)	0 (0)	0 (0)	0 (0)
Sterile bile	—	0 (0)	0 (0)	0 (0)	10 (0)
Pancreatitic bile	—	0 (0)	10 (10)	20 (20)**	30 (20)**
Infected bile	—	40 (40)*	40 (30)*	60 (50)*	90 (90)*

A.H.P. (mortality)

*$p < 0.01$

**$p < 0.02$

TABLE 11 Bile, infection and the pancreas: mortality and AHP rates

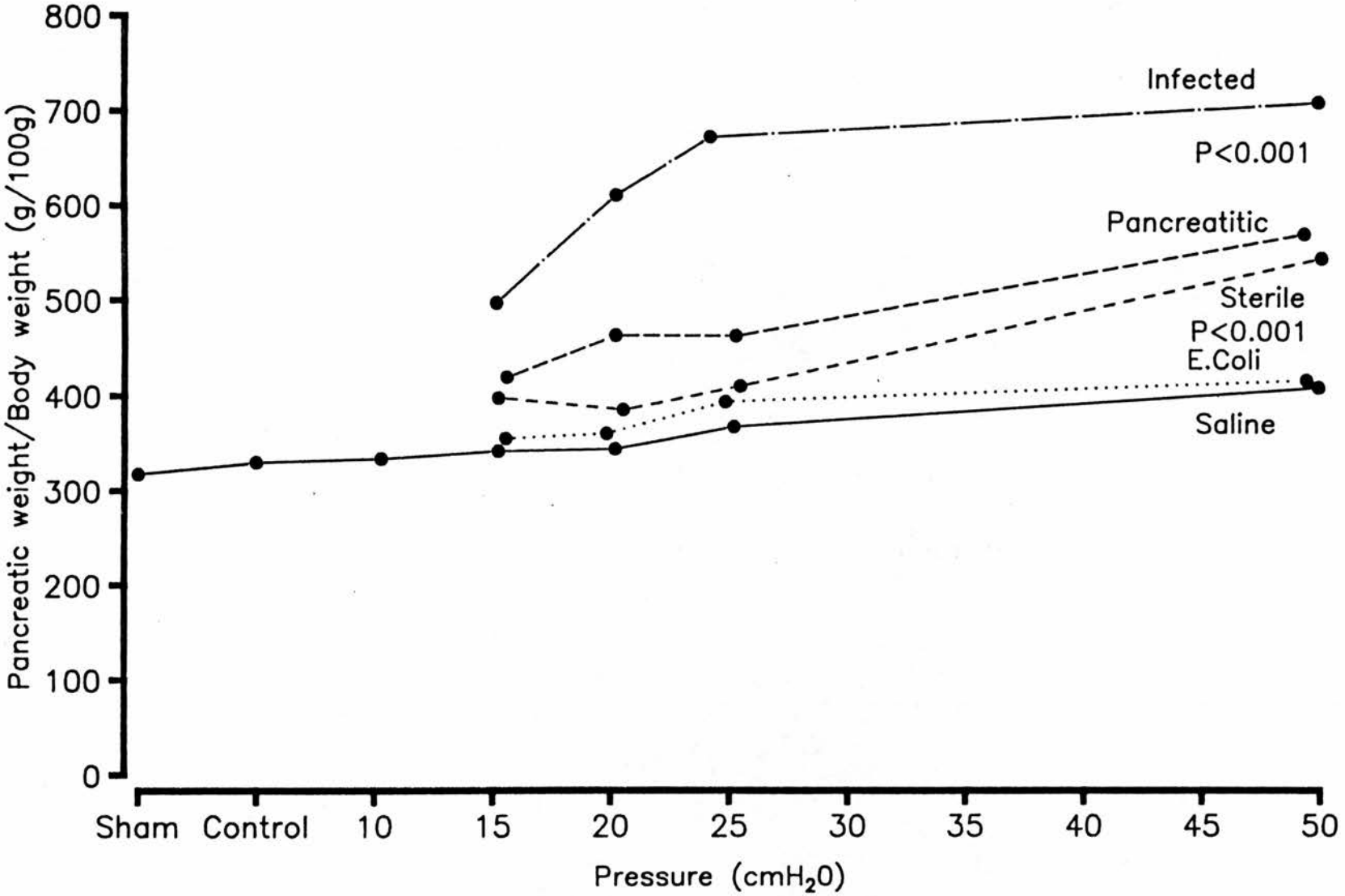


Fig. 20A Bile and infection vs. pancreatic gland weight ratio.

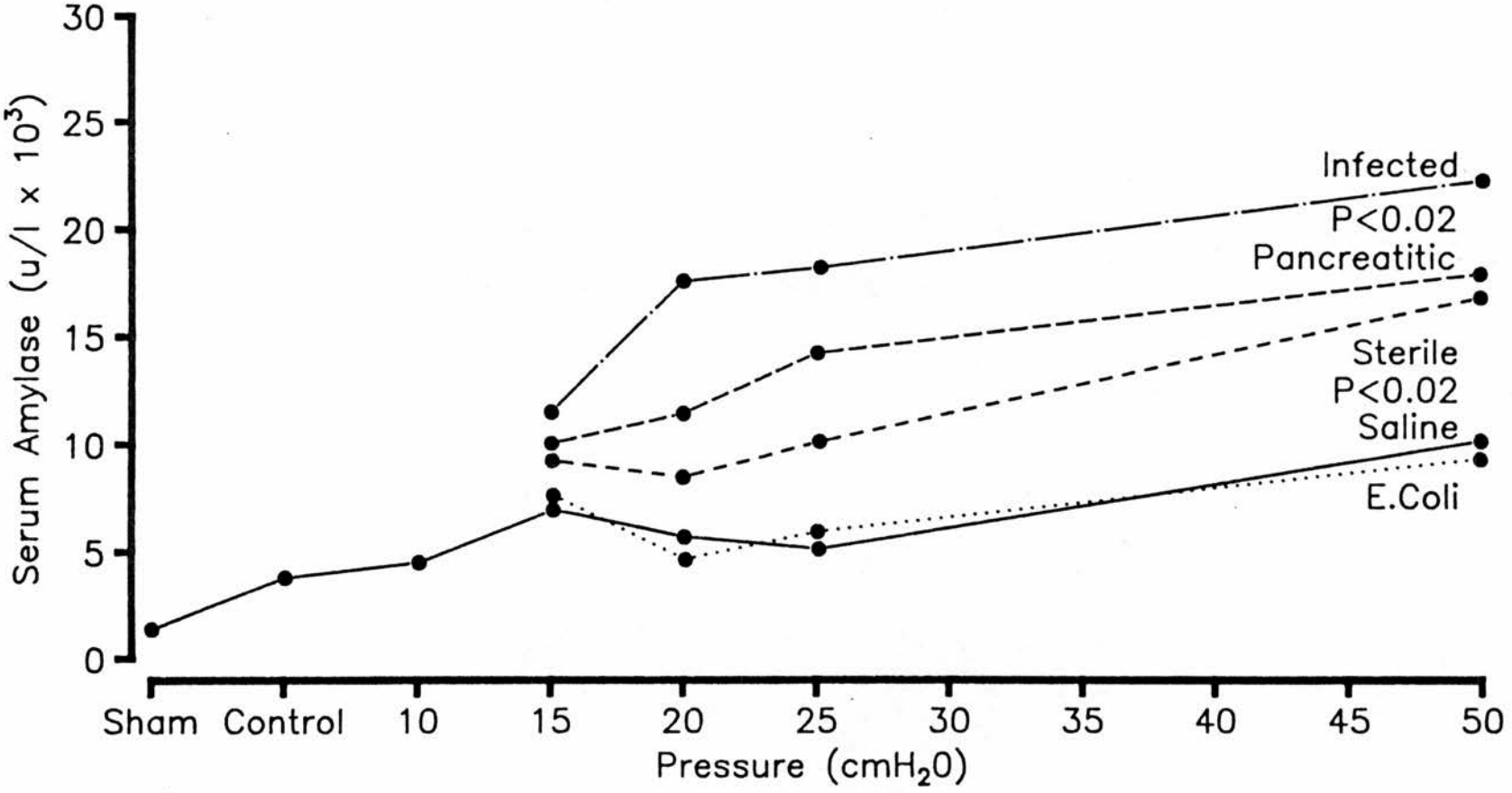


Fig. 20B Bile and infection vs. serum amylase.

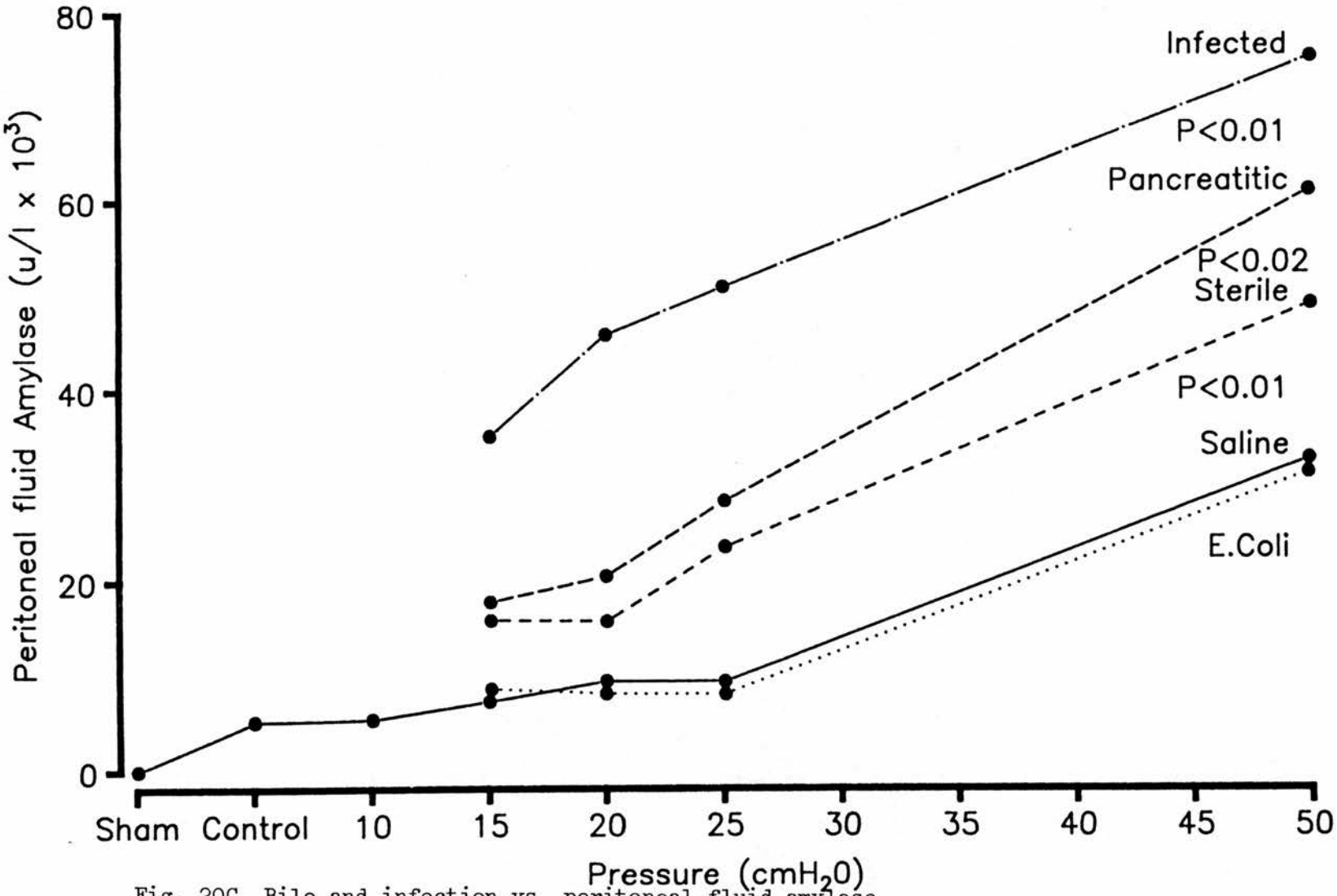


Fig. 20C Bile and infection vs. peritoneal fluid amylase.

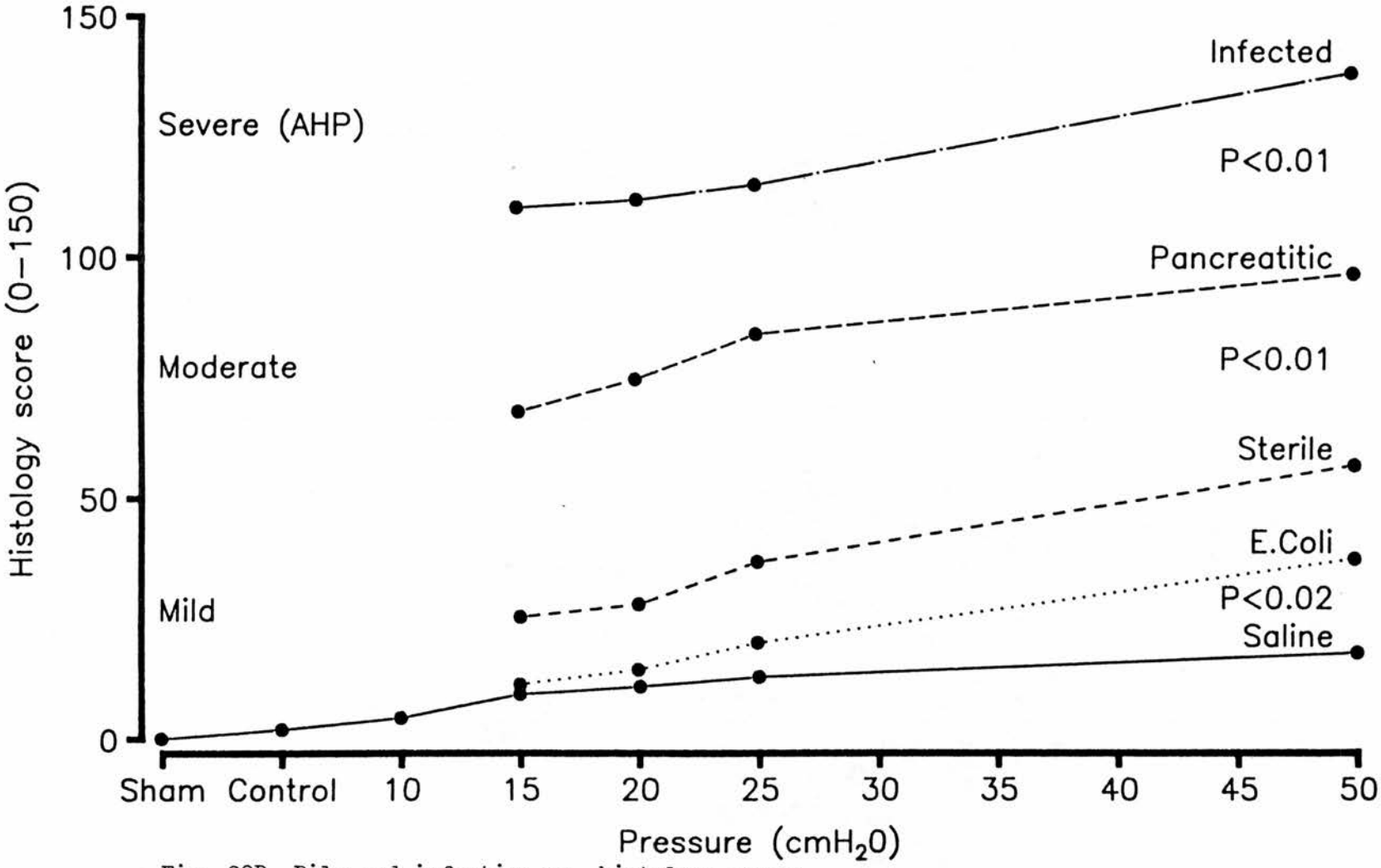


Fig. 20D Bile and infection vs. histology score.

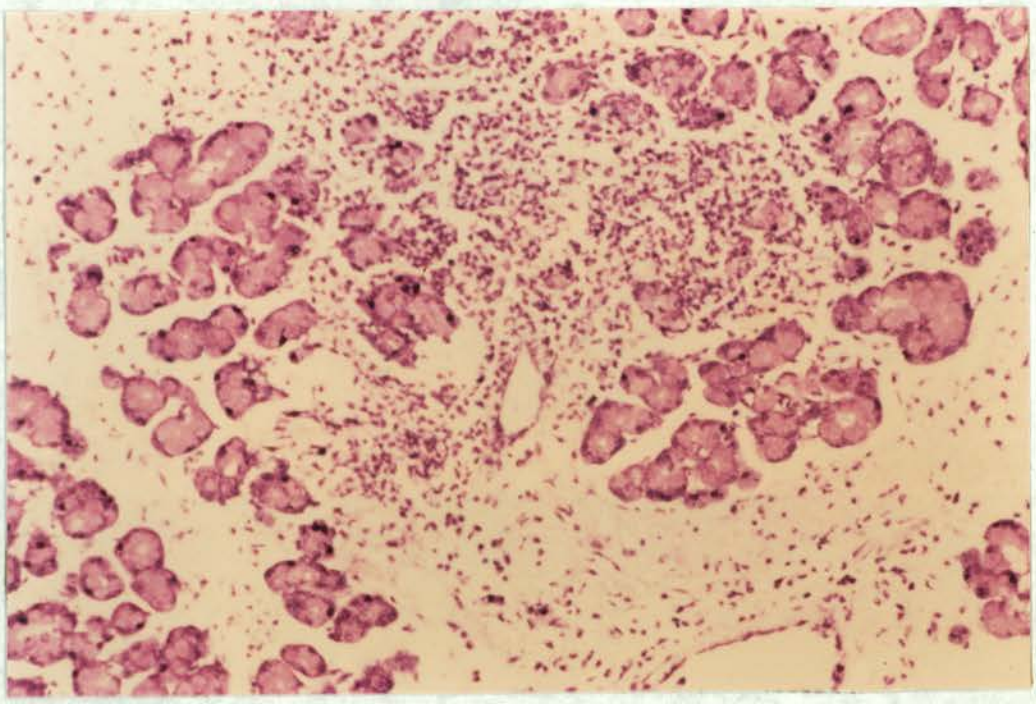


Fig. 21A Moderate pancreatitis (x 150).

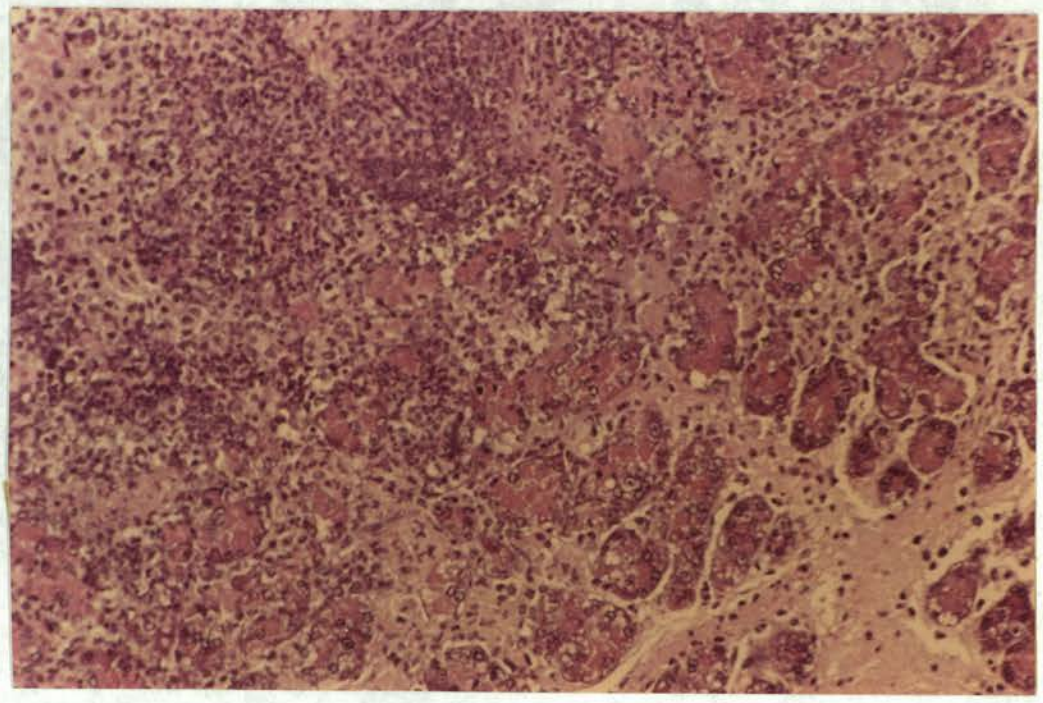


Fig. 21B Severe haemorrhagic pancreatitis (x 150).

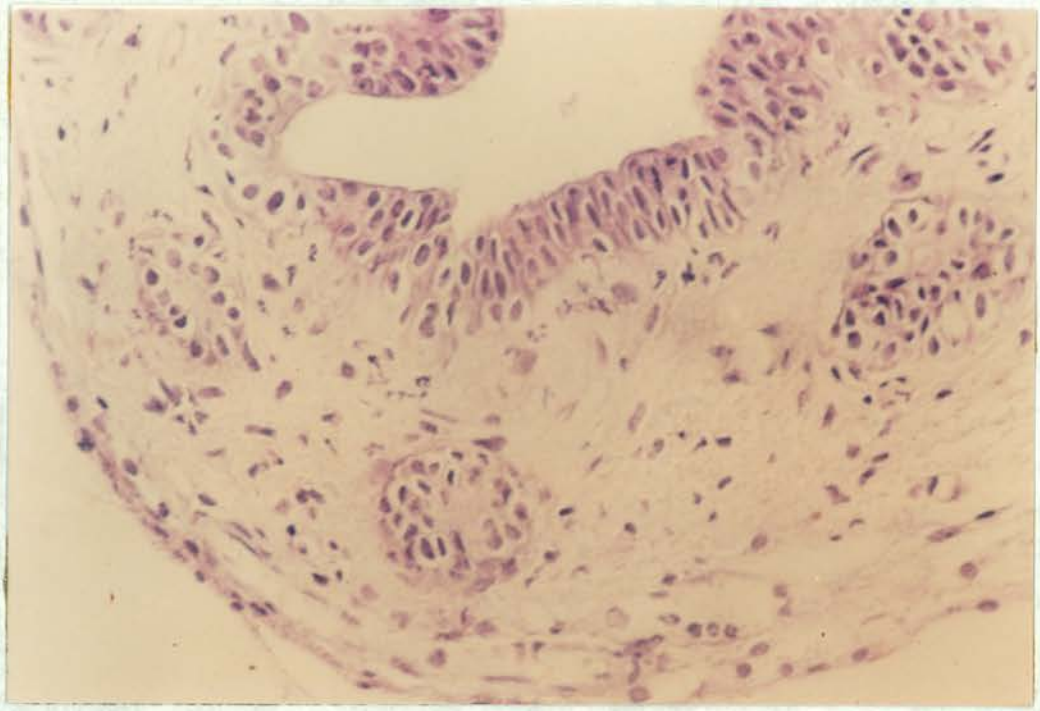


Fig. 22A Normal duct (x 300).

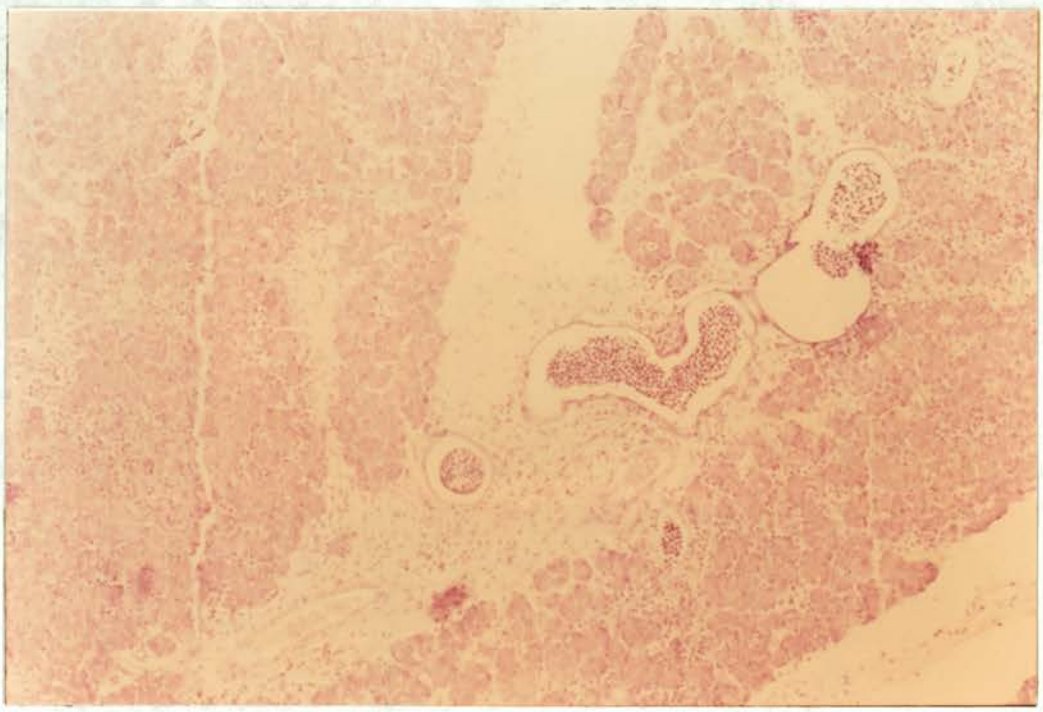


Fig. 22B Ducts filled with acute inflammatory cells (x 140).

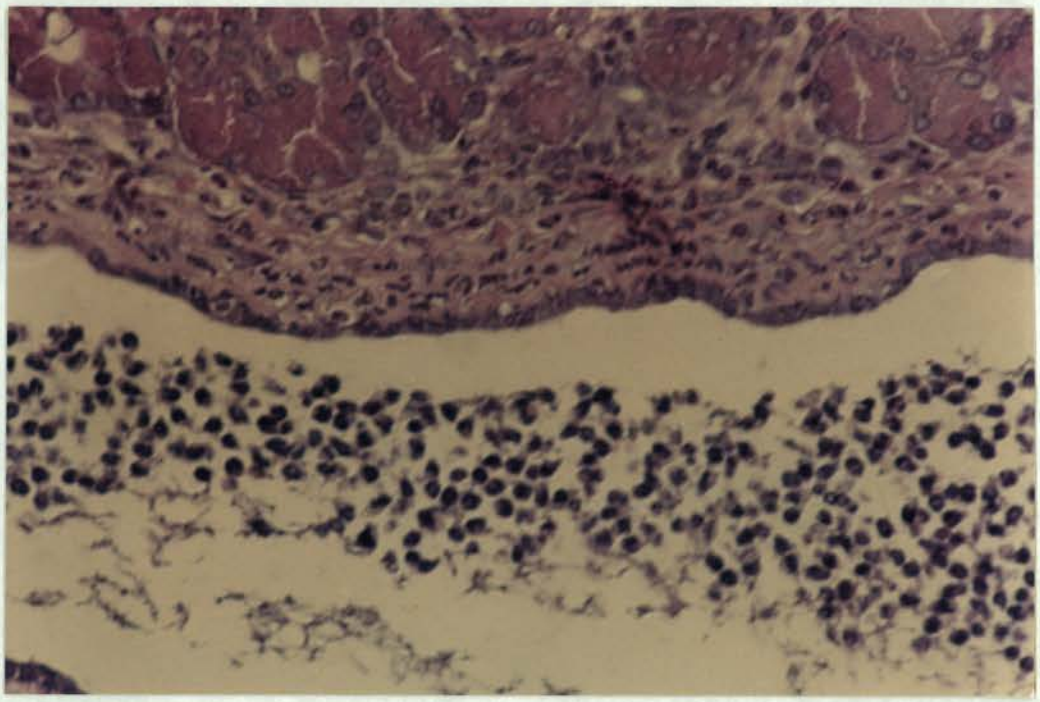


Fig. 22C Duct showing moderate inflammation (x 250).

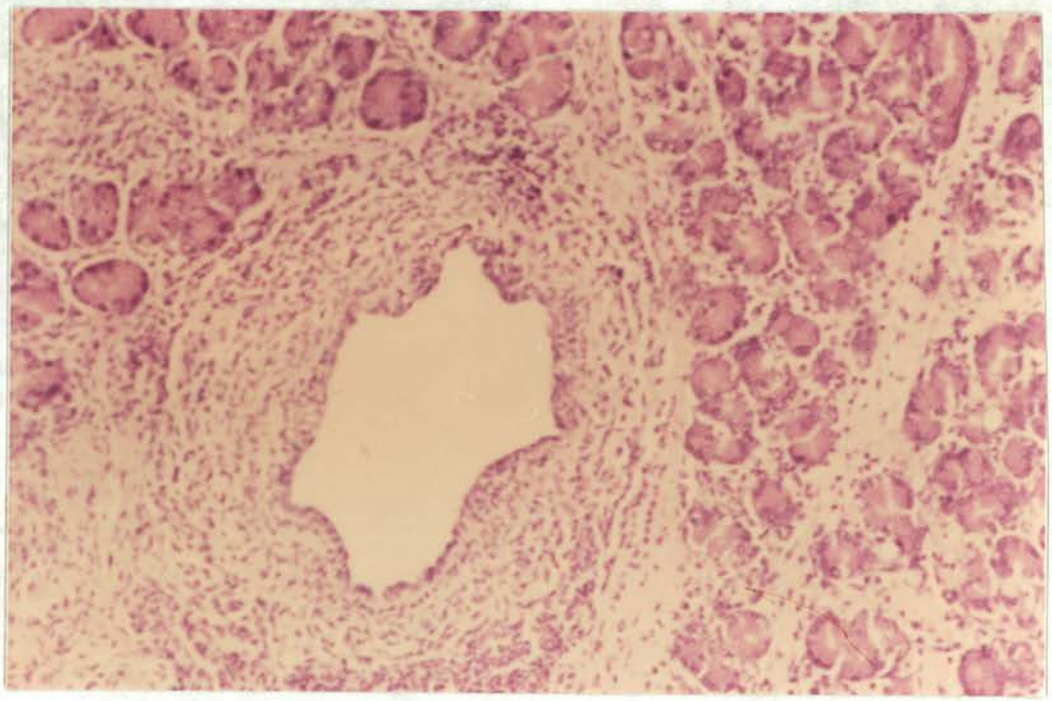


Fig. 22D Duct showing severe inflammation (x 200).

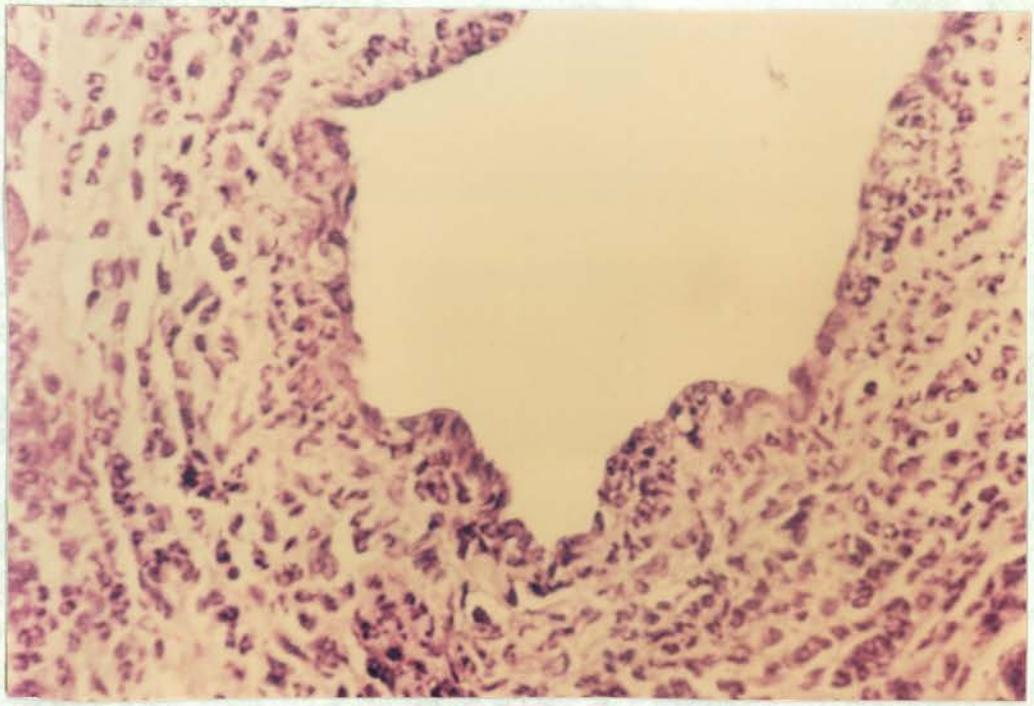


Fig. 22E Duct showing severe inflammation (x 250).

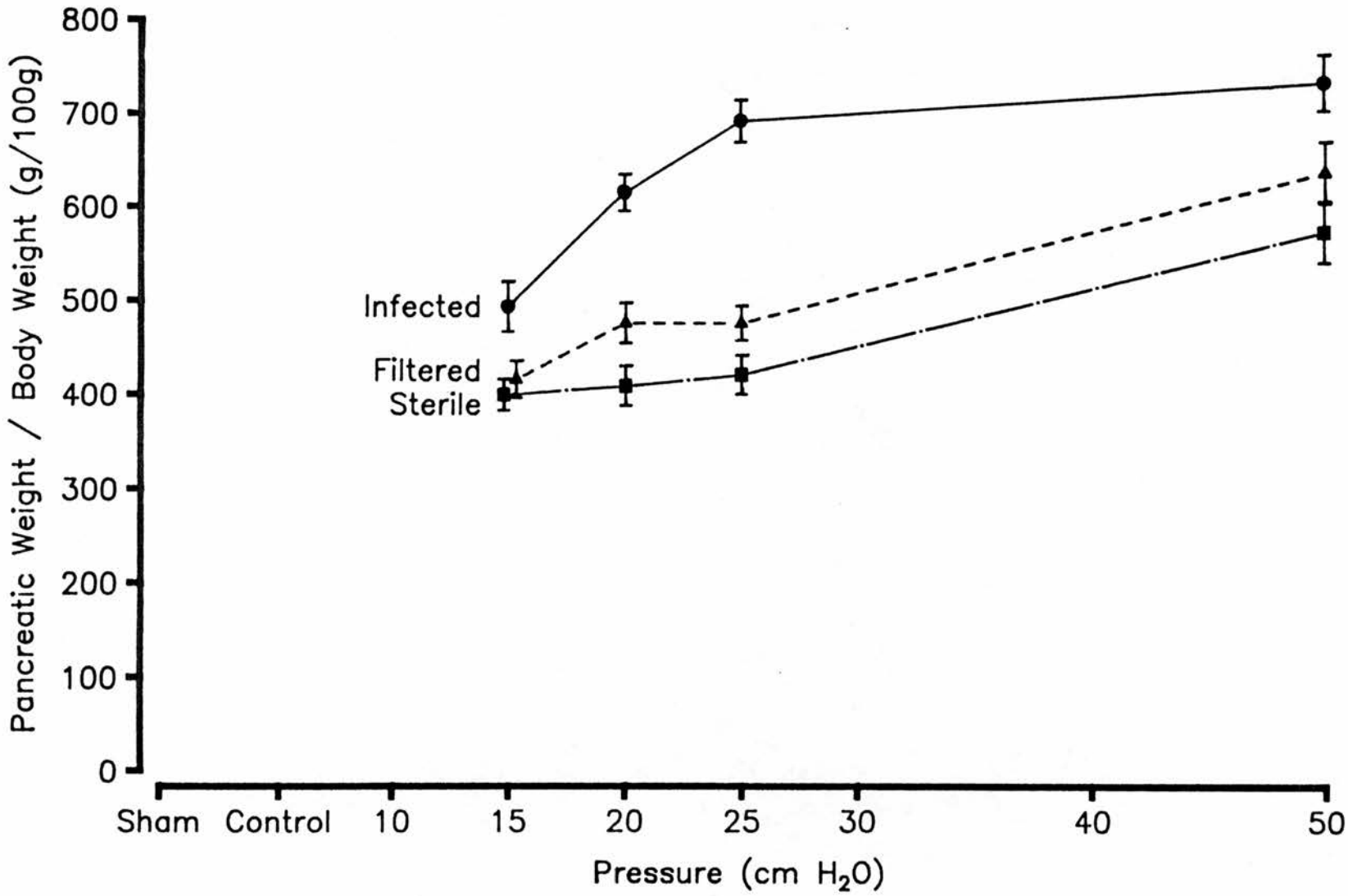


Fig. 23A Filtered bile vs. pancreatic gland weight ratio (mean ± SD).

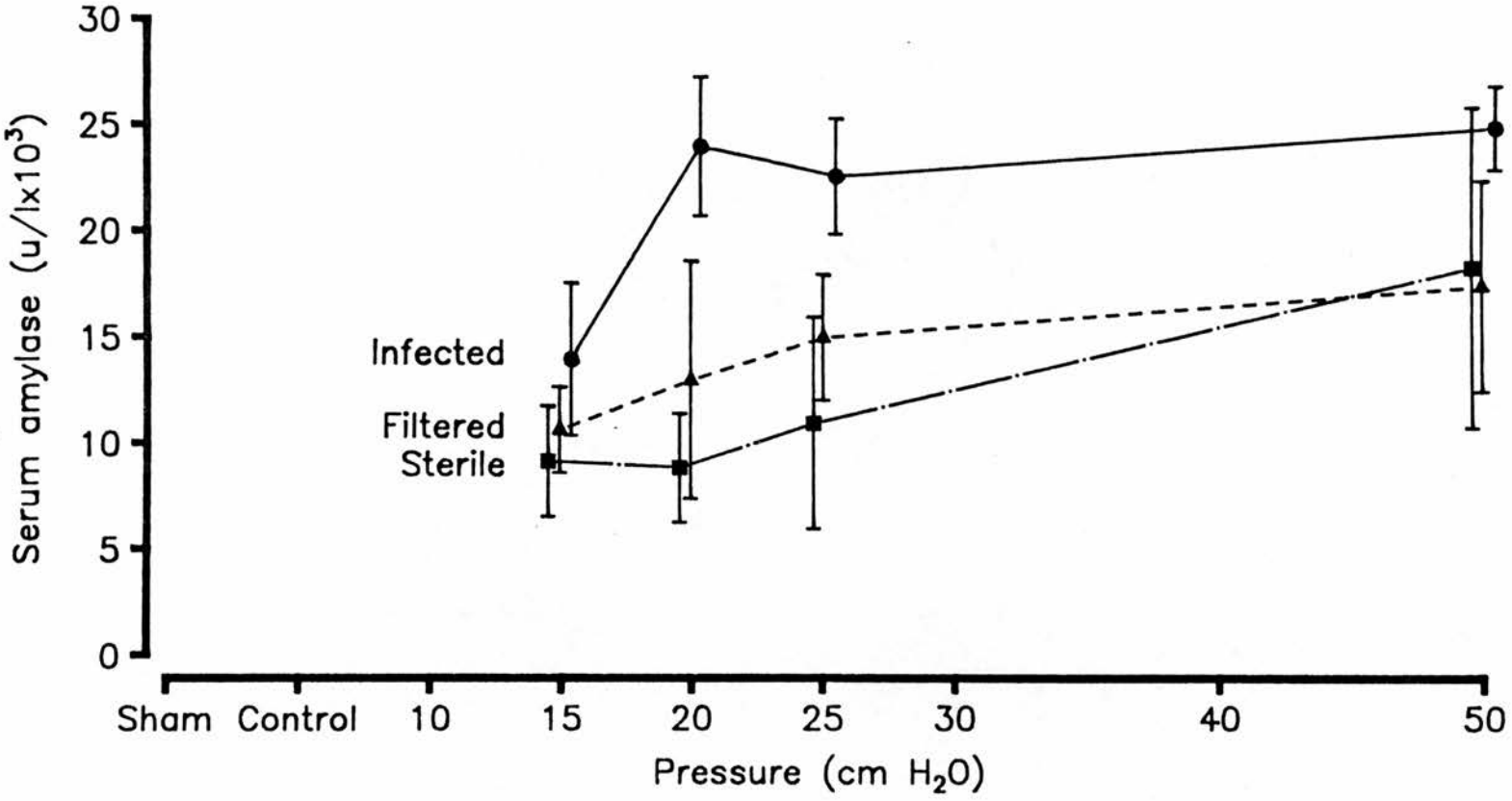


Fig. 23B Filtered bile vs. serum amylase (mean ± SD).

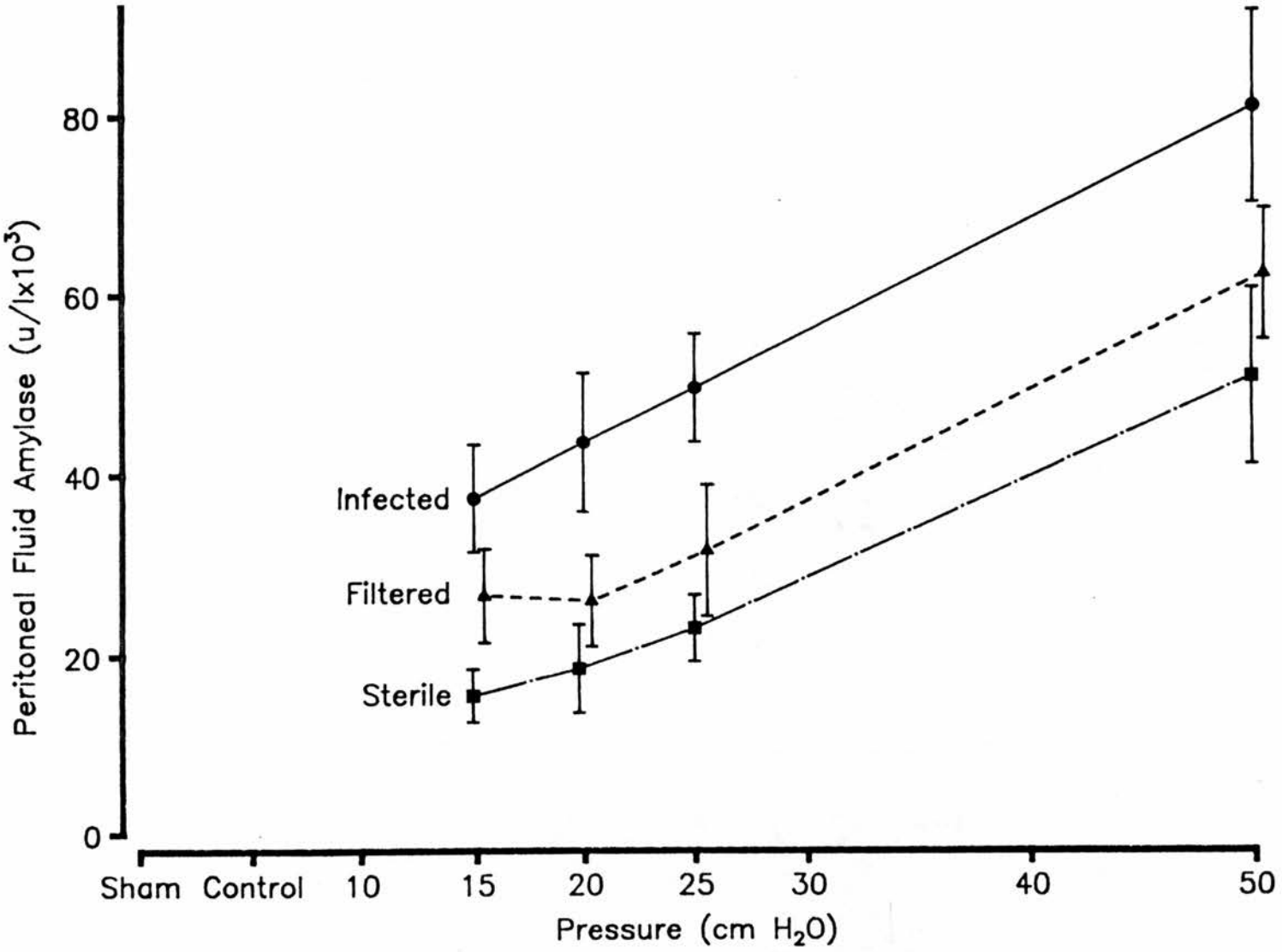


Fig. 23C Filtered bile vs. peritoneal fluid amylase.

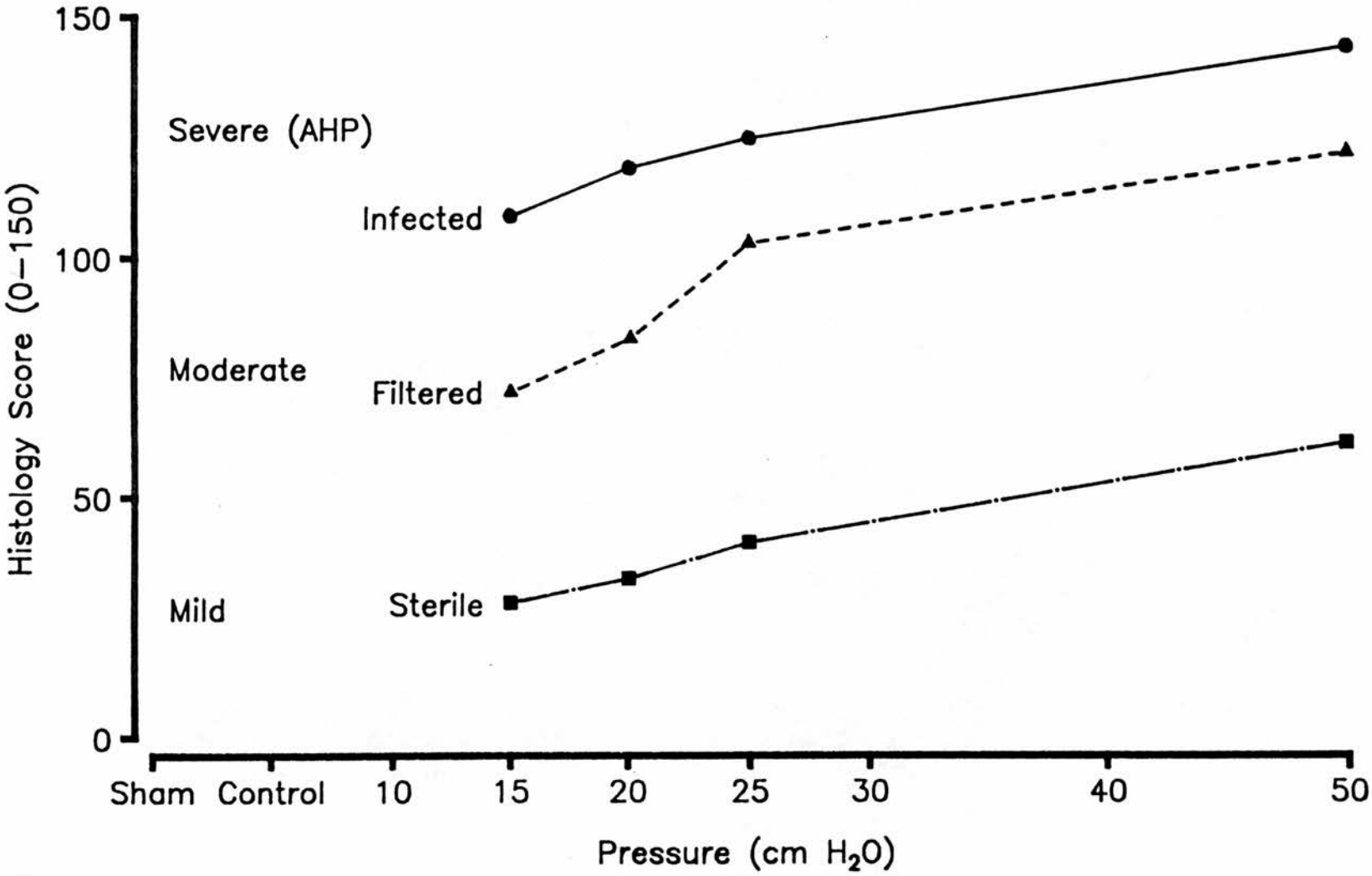


Fig. 23D Filtered bile vs. histology score.

Discussion

The rat is the only animal in which the large number of experiments sufficient for statistical anlysis are feasible on economic grounds. Furthermore the rat appeared to be an excellent animal for the study of the effects of bile on the pancreas since the pathological changes observed were similar to those described in man by Baggenstoss (1973) and Banks (1979). The previously described methods of determining pancreatic damage corresponded well together and appeared to be sensitive indicators of this damage.

Infusion of a freshly prepared bacterial solution (E. Coli), with proven viable biliary organisms, was little different to the infusion of saline alone. Indeed the only difference observed, of more marked histological change at high pressure, can be explained by rupture of the ducts and escape of the bacteria into the interstitium with the resultant inflammation. E. Coli bacteria alone have little effect on the pancreas but require a substrate such as bile. These results are in agreement with Mizumoto (1971) who found that β-glucuronidase from E. Coli was harmless alone but very toxic when added to bile. He showed that β-glucuronidase alone produced a reduction of the thin mucus layer of the pancreatic ducts, a feature not specifically examined in this study. The author was unable to confirm the cytotoxic effects on pancreatic ductal cells described by Keynes (1981) or the production of acute haemorrhagic pancreatitis (Thal 1956).

Sterile bile collected from 10 patients undergoing elective cholecystectomy was infused into the rat pancreas. The results obtained showed that pancreatic damage at all pressures was more marked than

after saline alone. At pressures, which were previously shown to be associated with extravasation through intercellular clefts, there was only mild to moderate pancreatic damage. At higher pressures with known duct rupture there was evidence of moderate damage but only one animal developed acute haemorrhagic pancreatitis. These observations confirm that sterile bile at low pressures does not cause pancreatitis (White 1960, Robinson 1963, Banks 1971). The results of high pressure infusion of bile suggest that pancreatic inflammation can occur once the bile extravasates from the duct into interstitial spaces below acinar tissue (Banks 1971). Therefore significant pancreatic inflammation can occur when sterile bile is under pressure and these results support the postulates of previous authors (Gilsdorf 1967, Sum 1970, Rittenbury 1969, Keynes 1981).

The investigation of the toxic properties of bile from patients with a recent attack of acute gallstone pancreatitis is a new concept, although ideas of "toxic" bile have been previously hypothesised (Hansson 1967, Banks 1971, Braganza 1983a,b, Parks 1983). The bile was obtained from four patients undergoing early cholecystectomy for acute gallstone pancreatitis. This "pancreatitic" bile appeared to have a lower pH than either the sterile or infected bile and no previous author has commented on this feature. However as the number of bile specimens tested was small this observation deserves future clarification with much larger numbers of specimens. "Pancreatitic" bile was more toxic to the rat pancreas than sterile bile after infusion at corresponding pressures. Several animals developed fatal acute haemorrhagic pancreatitis and this further evidenced the increased toxicity of "pancreatitic" bile. It is tempting to postulate several reasons for this increased pathogenicity. Possible explanations

could be: a change in the bile acid pool or phospholipid concentration or a relative change in their various constituents. The suggestion of Braganza (1983a,b) of a toxic metabolite in the bile cannot be discounted. As bile often refluxes into the pancreatic duct without subsequent damage it may be that patients who develop acute gallstone pancreatitis have an altered bile composition which produces the initial ductal damage. This result is an original observation which opens up possibilities for future research. There is need for a full biochemical and physical analysis of bile comparing the bile of patients with pancreatitis with that of patients without pancreatic disease but with gallstones. This analysis could explain why only 5% of patients with gallstones develop acute pancreatitis.

This study has shown infected bile to be far and away the most toxic to the pancreas. At all pressures most animals developed fatal acute haemorrhagic pancreatitis and this suggests that infected bile damages ductal epithelium and allows escape from the ducts independent to pressure. These results are in agreement with Konok and Thompson's (1969) observations on the cytotoxicity of infected bile to the pancreatic ductal epithelium. E. Coli was the organism most commonly grown from the samples of infected bile as reported by earlier authors (Suzuki 1984, Keighley 1978, Lennette 1980). The pH of infected bile appeared to be similar to that of sterile bile and was significantly higher than that of "pancreatitic" bile. When the same bile was filtered, removing all bacterial organisms, there was a reduction in its toxicity, confirming Keynes (1980) observations on the filtration of duodenal loop fluid. The pancreatic damage observed, however, was still worse than that seen with sterile bile. These results suggest that the toxicity of infected

bile could be due to (i) the organisms themselves interacting with bile and (ii) bacteria producing toxins (e.g., lecithinase, β-glucuronidase) or altering the composition of the various biliary constituents. The effect of infected bile on the pancreatic duct mucosal barrier is discussed in Chapter 9. Bile infected with pseudomonas organisms was extremely toxic to the pancreas as all animals developed a particularly haemorrhagic form of acute pancreatitis. Pseudomonas produces elastase, an enzyme which has been implicated in the production of vascular injury during human acute pancreatitis (Geokas 1968a,b, 1969, Wanke 1970). Further study of the role of elastase and its relation to bacteria appears merited.

This study of the effects of bile and infection on the pancreas is the first to take careful note of both the volume and pressure of injection. Only when these two factors are carefully controlled can extrapolation of the results of animal studies be made to the clinical situation in man. Indeed many previous authors have made definitive statements regarding the pathogenicity of bile to the pancreas. A careful review of their experimental methods reveals that the pressure of injection was too high or the volume grossly in excess of that likely to reflux in man, making interpretation very difficult. Other important points to consider when investigating the effects of bile are that bile itself varies considerably between individuals and that animal susceptibility varies within the same species. For these reasons this study has used bile from a number of similar patients and investigated its effect on groups of ten animals. This study also fulfills the criteria of Elliott (1976) in producing meaningful lesions in the pancreas whereby the pressure is controlled, a physiological

volume of solution is used and the time which the solution is applied is carefully measured.

Conclusions

1. A bacterial solution of E. Coli was no more pathogenic than saline to the pancreas.
2. Sterile bile produced pancreatic inflammation which was only significant when the pressure of infusion was high.
3. "Pancreatitic" bile has unique properties that made it more toxic to the pancreas than sterile bile.
4. Infected bile was extremely noxious to the pancreas at all pressures.
5. Filtration of infected bile partly reduced its pathogenicity suggesting a role for both bacteria and their toxins or a change in biliary composition.

CHAPTER VI

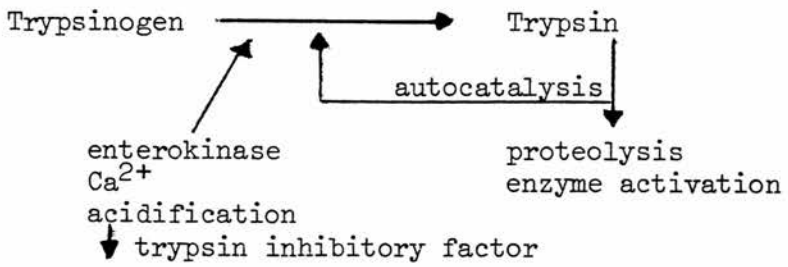
TRYPSIN, ENTEROKINASE AND BILE SALTS AND THE PANCREAS

Introduction

Acute pancreatitis is considered to be a chemical autolysis of the pancreas triggered by the activated pancreatic enzyme trypsin (Ohnishi 1984). There is, however, conflicting opinion regarding the role of trypsin, enterokinase and bile salts in the initiation of acute gallstone pancreatitis. The theories of bile reflux via a common channel and duodenal reflux both take cognisance of the possible role of enterokinase, trypsinogen activation and bile salts from bile or the duodenum in the pathophysiology of acute gallstone pancreatitis.

Trypsin

Under normal conditions trypsin is produced by the acinar cell as a pre-enzyme, trypsinogen, which requires activation by enterokinase following its discharge into the duodenum. Trypsinogen exists as two proteins of 225 and 228 amino acids with molecular weights of 23,000 and 25,000. They are both activated by hydrolysis of a terminal hexapeptide to produce active trypsin by a number of substances (Desnuelle 1979) viz.



Trypsin is a particularly important enzyme as, apart from its role in protein digestion, it is responsible for activating the other pancreatic zymogens. Pancreatic juice also contains trypsin inhibitor which is of fundamental physiological importance. There are two inhibitors of trypsin present in pancreas. The first is the Kunitz (1936) inhibitor which inhibits both trypsin in a 1/1 ratio and other proteases. The second, and most important in man, is the "pancreatic secretory inhibitor" which is present in relatively large amounts (0.3 to 0.6 per cent of the total proteins of pancreatic juice) (Desnuelle 1979).

When considering trypsin as the primary mediator of pancreatic inflammation it is important to evaluate the mechanism by which this protease is converted from precursor to active form (Beck 1962, Anderson 1969). Fresh pancreatic secretions obtained from pure pancreatic fistulas contain little or no proteolytic activity and Anderson (1969) has postulated that trypsinogen is converted to an active form when the enzyme passes into the interstitium of the gland during periods of ductal obstruction. Since there are trypsin inhibitors within the pancreas and within pancreatic juice and trypsin itself is not activated until it comes into contact with enterokinase within the duodenum, consideration has been given to the concept that reflux of duodenal contents into the pancreatic duct is essential for the development of pancreatitis (McCutcheon 1968, Banks 1979). More recently however, several studies have shown that pancreatic enzymes may be activated without exposure to duodenal juice, and pancreatic juice of patients with acute pancreatitis has been documented to contain active trypsin, chymotrypsin and elastase (Geokas 1974). The role of activated trypsin in causing pancreatitis has been substantiated as active trypsin

has been found within both the pancreas and pancreatic exudate during experimental pancreatitis (Ohlsson 1975, Rao 1976, Herva 1970). Thus trypsin can be activated under certain circumstances without prior entry into the duodenum.

The effect of a retrograde injection of trypsin into the pancreatic duct of experimental animals depends on the amount given (Creutzfeldt 1970). Small amounts lead only to transitory interstitial oedema whereas large doses effect impressive morphological changes (Beck 1964). Anderson and colleagues (1969) from Toledo, Ohio have studied the effects of enzymes on the pancreas. They postulated that enzymes back-diffuse into the interstitium of the gland through minute clefts between cells (as discussed in Chapter IV). When trypsinogen was infused into the pancreas there was little resultant inflammation whereas fresh active trypsin produced acute pancreatic inflammation and the magnitude of pancreatic damage was related directly to the amount of enzyme infused. Activated trypsin or chymotrypsin, infused into the canine pancreatic duct, causes rapid accumulation of oedema fluid in the interlobular spaces (Anderson 1962). The intensity of this response appears to be dose-related; increasing amounts of trypsin produce lesions of greater intensity, converting simple oedema to a serosanguineous exudate or even gross haemorrhage.

Aho and co-workers (1982) studied the light and electron microscopic characteristics of early pancreatic lesions produced by intraductal injection of trypsin, using high pressures, volumes and supraphysiological doses. The changes identified were those of: swollen cells, necrosis, degeneration of nuclei and vacuolation of cytoplasm. These areas of

necrosis were similar to those described after trypsin injection by Beck (1971) and Baggenstoss (1973). Indeed Beck (1971) made careful distinction between the cytolytic necrosis of trypsin and the coagulation necrosis of bile salts. Trypsin may initiate pancreatic damage primarily by activating elastase, phospholipase A and possibly Kallikrein rather than by attacking normal pancreatic tissue directly. Intraductal activation of trypsinogen and leakage into the interstitium might occur when intraductal pressure rises.

The effects of trypsin inhibitors on the course of acute pancreatitis have been the source of much debate since aprotinin (trasylol) was introduced (Trapnell 1974, MRC study 1976). Recently Takasugi (1982) and Ohnishi (1984) have demonstrated the efficacy of both synthetic and naturally occurring trypsin inhibitors in ameliorating acute experimental pancreatitis. They suggested that trypsin inhibitor might act by preventing the chain reaction of activation of pancreatic enzymes. In all these studies, however, trypsin inhibitor was given at the time of induction of pancreatitis; a situation hardly pertinent in the human clinical setting.

What then is the role of trypsin in inducing pancreatic damage? Low dose trypsin infused intraductally gives oedema only, whereas a high dose of the protease produces necrosis. Reflux of duodenal or biliary constituents cause activation of the zymogen. An increase in intraductal pressure leads to extravasation into the interstitium where active trypsin exerts its pathological effects. The effects of small physiological doses of trypsin given, in a small volume at low pressure, on the pancreas are to date unknown.

Enterokinase

Human enterokinase is a large molecule with a molecular weight of about 300,000 (Grant 1983, 1976). It is particularly resistant to proteolysis (Grant 1976, Yamashira 1956) and is one of the most heavily glycosylated enzymes known (Magee 1981). In man enterokinase has been localised to the brush border of the enterocytes lining the duodenum and the first 10-20 cm of jejunum (Hermon-Taylor 1977, Grant 1981).

Mann and colleagues (Hammond 1977) have demonstrated that injection of active enterokinase into the canine pancreatic duct produces hyperamylaseamia and acute pancreatitis. Inactivation of enterokinase or administration of trypsin inhibitor prevented pancreatitis but not the rise in amylase levels. The lowest concentration of enterokinase which produced pancreatitis was 0.5% - equivalent to 1-2 ml of duodenal fluid, which is a volume well within the range of possible duodenal reflux. Mann (1979, 1969, 1977) further showed that intraductal injection of 1% enterokinase into rats produced acute pancreatitis. He was careful to control pressures to 8 cm H_2O but used a volume of 1 ml, previously shown to produce widespread ductal rupture.

Hermon-Taylor and associates (Grant 1981, 1979, 1983, Terry 1982, 1983) have postulated that the biliary excretion of catalytically active enterokinase is the critical factor in the development of some forms of acute haemorrhagic pancreatitis. They propose that an important event in the aetiology of acute pancreatitis is the transfer into bile of enterokinase, sequestered from the proximal small intestine into portal blood, and have evidenced this theory by showing that animals with liver

damage excrete two to four times more active enterokinase than normal. Terry (1982) studied the combined effects of enterokinase and bile salts in the development of murine acute pancreatitis. When either alone were given little damage resulted whereas infusion of a mixture of both resulted in a 100% mortality. This study used a relatively physiological 100 µl infusion. Unfortunately there was no record of intraductal pressure but the values given suggest a pressure of 40-50 cm H_2O with known duct rupture.

These studies indicate that enterokinase infusion causes pancreatic damage equivalent to that of activated trypsin injection. When enterokinase is combined with bile salt there is a potentiation of its pancreatotoxicity. The effects of physiological concentrations of enterokinase given in small volumes at low pressure remain as yet uncertain.

Bile salts

It has been postulated that the direct cytotoxic action of bile might be due to bile salts (Beck 1970, Reber 1979, Hansson 1967). The liver produces the primary bile salts cholic (CA) and chenodeoxycholic (CDCA) acid. These are subsequently converted in the bowel into the secondary bile salts, deoxycholic acid (DOC) and lithocholic acid, which are largely reabsorbed and secreted unchanged in the bile. The concentrations of bile salts in bile are summarised below.

	Bile Acid	mol wt	GB Bile	duodenum	
primary	(cholic	410	40 mM	2.3 mM	trihydroxy
	(CDC	393	40 mM	2.3 mM	)
					)
secondary	(Deoxycholic	393	16 mM	1.2 mM	)dihydroxy
	(Lithocholic	390	3.8 mM	0.03 mM	)

(Reber 1980)

In bile these bile acids are almost entirely conjugated with glycine and taurine in a 3:1 ratio. Free bile acids are rarely found except when bile is infected with bacteria capable of deconjugation (Hansson 1967; see Chapter V). Hansson (1967) studied the effects of different bile salts on the rat pancreas using the unphysiological volume of 1.5 ml. He showed that dihydroxy bile salts were 4 to 8 times as toxic to the pancreas as were the trihydroxy salts and free bile salts were twice as toxic as their conjugates. Since then there has been extensive investigation of experimental pancreatitis using a variety of bile salt preparations.

e.g. taurocholate - Aho 1980a,b, 1982, 1983, Lankisch 1983.
deoxycholate - Olazabal 1980, Greuter 1981.
glycodeoxycholate - Terry 1982, 1983.

Sum and colleagues (1970) investigated the possibility that bile might induce pancreatitis through the physicochemical detergent properties of its bile salts (Hoffmann 1965, Davenport 1968). As bile and bile salts produced similar lesions to those of the detergent sodium lauryl sulphate, they suggested that the toxic action of bile and bile salt was due to their anionic detergent property; an important factor in the early pathophysiology of bile induced pancreatitis. Bile acids in concentrations normally found in bile are destructive to pancreatic cells (Creutzfeldt 1970, Hansson 1967). According to Beck (1971) this is a detergent action producing coagulation necrosis. Bile acids may further liberate minute amounts of active trypsin and they serve as activators of phospholipase A (Creutzfeldt 1970).

Aho and associates (1980a,b) carefully studied the effects of sodium

taurocholate on the rat pancreas. Injection of 200 µl of sodium taurocholate produced acute haemorrhagic pancreatitis and the mortality increased with the amount of bile salt injected. Pancreatic lesions were immediate and characterized by interstitial oedema, extensive necrotic changes in the acinar cells and haemorrhages during the first 24 hours after the injection. The first histological change in acute bile salt- induced pancreatitis was dissolution of the duct walls, with destruction of adjacent lobules. These investigators demonstrated that the presence of a bile salt within the duct system of rat pancreas could initiate a disease with gross and histopathological changes corresponding to those seen in human acute pancreatitis (Baggenstoss 1973). Aho (1980b) further examined the pancreatic damage by means of electron microscopy. The earliest cell injury response (1 minute after injection) was characterized by the complete dissolution of cellular membranes and a granular appearance in the cytoplasm, changes attributed to the detergent action of the bile salt. Later changes indicated enzymatic autodigestion of the acinar cells. These studies can be criticised for the large volume of infusate and the very high pressures used as in these conditions there is complete duct disruption and extravasation into the interstitium. Thus, whilst bile salts are indeed toxic to the whole pancreas, their major toxicity is by way of detergent action on ducts and cell membranes. This effect on the duct will be studied later in relation to the "pancreatic duct mucosal barrier".

Heuman (1980) has recently shown that bile from patients with cholesterol gallstones contains relatively more deoxycholates (19.4%±8.6%) than that of control patients (14.1%±6.0%) whereas the proportions of cholic and chenodeoxycholic acids remain relatively constant. Indeed, it has been

proposed that alterations in the chemical composition of gallbladder bile may play some role in the pathogenesis of acute cholecystitis. The nature and relative proportions of bile salts in patients with acute gallstone pancreatitis remains uncertain.

Several conclusions can be drawn from these studies on bile salts.

(i) bile salts act as detergents with an immediate membrane toxicity to the duct walls.

(ii) Different bile salts have differing toxicity to the pancreas i.e. DOC > CDC > CA. Free bile acids are even more toxic.

(iii) Patients with gallstones have differing bile salt concentrations in their bile. The nature of "pancreatitic" bile is unknown.

(iv) Injection of bile salts causes acute haemorrhagic pancreatitis, although the volumes and pressures hereto used have been unphysiological.

Trypsin and Bile salts

A mixture of trypsin and bile salts is extremely toxic to the pancreas (Elliott 1971, 1968, 1957, Elmslie 1966, Orda 1980, Doerr 1965). Elliott and colleagues (1957), in a classic study reported from Ohio State University, evaluated alterations in the pancreatic resistance to bile in the pathogenesis of acute pancreatitis. They found that bile or pancreatic juice alone produced moderate to pancreatic damage. A fresh mixture of these two fluids was slightly more toxic. When, however, bile and pancreatic juice were incubated for 12 hours there was a marked increase in toxicity; more could be infused into the pancreas at a given pressure and there was a 100% mortality from haemorrhagic pancreatitis.

It appeared as though bile had undergone a fundamental change during its incubation with pancreatic secretion. Furthermore, trypsin alone was capable of producing the same changes as pancreatic juice, and the most toxic mixture was a 1:1 ratio of the two fluids. In the context of a gallstone causing temporary obstruction of the ampulla of Vater such mixing might occur.

In contrast to these findings Anderson and associates (1958) demonstrated that a combination of trypsin and bile salts did not appear to produce a lesion in any way superior to that observed when bile salts alone were used. Elmslie (1966) determined whether a "physiological" volume of bile and trypsin would affect the pancreas when introduced into the duct. He reviewed earlier reports on the production of fatal pancreatitis by the injection of solutions into the canine pancreatic duct and found that the volumes employed were over six times the capacity of the adult dog pancreatic tree - i.e. the production of pancreatitis by these methods appeared to have no application in the pathogenesis of the human disease. His experiments demonstrated that a fresh mixture of dog gall-bladder bile and human trypsin was as effective as bile alone in producing pancreatitis. The role of trypsin and bile salt, when admixed, in the pathogenesis of acute gallstone pancreatitis remains unclear. Most experiments to date have used volumes and pressures grossly in excess of those likely to occur in the physiological setting.

What conclusions can we draw from these various studies on the role of trypsin, enterokinase and bile salts in the pathogenesis of acute gallstone pancreatitis?

I. Trypsin and enterokinase alone produce oedematous change only.

II. Bile salts are primarily attackers of membranes and may have an important early role in the initiation of gallstone pancreatitis.

III. Mixtures of trypsin, enterokinase and bile salts are probably more toxic than when each is given alone.

There are many "grey areas" in this field and our knowledge of the role of these three compounds in the pathogenesis of AGP remains sketchy. Indeed, what effect do these compounds have on the pancreas when given at physiological volumes and pressure? These observations prompted us to study the effects (at physiological volume and pressure) of trypsin, enterokinase and bile salt on the rat pancreas.

Material and Methods

Object

To establish the role of trypsin, enterokinase and bile salts, individually and in combination in the pathogenesis of acute gallstone pancreatitis. The effects of pressure and volume were carefully assessed.

Experimental preparation

The experimental preparation described in fig. 5 was used throughout this study. A solution containing trypsin, enterokinase or glyco-deoxycholate was infused into the pancreatic duct at varying pressures of 15, 20, 25, 50 cm H_2O. Cannular occlusion in this experiment was for 5 minutes only. The animals made a full recovery until sacrifice at 24 hours with pancreatic damage being assessed by gland weight, serum and peritoneal fluid amylase levels and the histology score. Groups of eight rats were used throughout the experiment.

Solutions: (50 µl volume used throughout).

Trypsin. (Sigma, T-8642, from bovine pancreas; 12,100 BAEE units/mg protein) (Benzyl arginine ethyl ester)

A fresh solution was made up by adding trypsin to sterile 0.9% saline to give a concentration of 300 u trypsin/50 µl saline. The activity of trypsin was tested before use by its action on BANA (Huttenen 1973). (Benzyl arginine nitroanilide)

Enterokinase: (Sigma, E-1256, from procine intestine; 8.3 units/mg protein; 1 unit activates 0.065 mg trypsinogen per hour at pH 5.8 at 5°C (Kunitz 1939)). A fresh solution was made up before each experiment by adding enterokinase to 0.9% saline. The concentrations of

enterokinase was either high (200 µg/50 µl) or low (200 ng/50 µl).

Glycodeoxycholate (GDC): (Sigma, G-3258, sodium salt, mol. wt. 471.6). A 10 mM solution was freshly prepared before each experiment by adding glycodeoxycholate to 0.9% saline.

combinations

(i) glycodeoxycholate (10 mM) + enterokinase (200 ng) in 50 µl saline.

(ii) glycodeoxycholate (10 mM) + trypsin (300 u) in 50 µl saline.

(iii) trypsin (300 u) + enterokinase (200 ng) + glycodeoxycholate (10 mM) in 50 µl saline.

All combination solutions were freshly prepared before use.

Results (summarized in tables 12 to 15).

Trypsin

Immediately on injection the pancreas became extremely oedematous but no haemorrhage was noted. All animals survived to 24 hours. At sacrifice the macroscopic appearances were those of marked oedema with a moderate quantity of ascitic fluid and a few fat necroses. The appearances were those of mild to moderate pancreatitis. At all pressures the PGWR was significantly higher than with saline infusion alone ($P < 0.01$) and the highest PGWR was obtained at a pressure of 50 cm H_2O. Serum amylase levels were similar to those of saline infusion whereas the peritoneal fluid amylase was significantly elevated at pressures of 20 cm H_2O and above. The histological changes produced by trypsin infusion were more marked ($P < 0.01$) than those of saline. There was evidence of moderate oedema with inflammatory cell infiltration although little acinar necrosis and no haemorrhage or duct damage were apparent. In no animal was there evidence of acute haemorrhagic pancreatitis. Trypsin therefore gave a moderate oedematous or interstitial pancreatitis with the most marked changes noted at higher pressures of infusion.

Enterokinase (high and low dose)

Infusion of high dose enterokinase produced moderate pancreatic oedema within 2-3 minutes; low dose produced little effect. All animals survived to 24 hours and appeared to be well at that stage. At sacrifice the pancreas of the low dose infusion group had a macroscopic appearance indistinguishable from that of saline only i.e. moderate oedema and little peritoneal fluid. High dose enterokinase produced moderate oedema with peritoneal fluid and several fat necroses. No haemorrhage was seen.

The PGWR was significantly higher for enterokinase infusion at all pressures than saline alone. The most marked differences were seen with the high dose. The gland weights obtained with the high dose enterokinase infusion were very similar to those of trypsin alone. Serum and peritoneal fluid amylase levels were not significantly elevated with a low dose of enterokinase. In contrast high dose enterokinase produced marked ($P<0.01$) elevations of both PFA and SA. The changes in peritoneal fluid amylase were of a similar magnitude to those seen with trypsin whereas serum amylase levels were twice as high. Low dose enterokinase produced histological evidence of moderate oedema only; of a more marked degree than saline alone ($P<0.02$). High dose enterokinase produced severe oedema with a minimal inflammatory infiltrate. In no animal were there duct changes, evidence of acinar necrosis or haemorrhage.

High dose enterokinase produced pancreatic damage of a similar magnitude and character to that seen after trypsin infusion.

glycodeoxycholate (GDC)

Infusion of glycodeoxycholate solution produced changes of a different nature to those seen after trypsin and enterokinase. All animals survived to 24 hours. On injection there was little obvious signs of pancreatic damage in contrast to the immediate oedema produced by trypsin and enterokinase. At 24 hours mild to moderate pancreatitis was noted with the most marked changes being those of oedema, ascites and fat necroses; little haemorrhagic change was seen. The PGWR was markedly elevated compared with saline alone ($P<0.001$) and the values obtained were similar to those seen after trypsin or high dose enterokinase infusion.

The highest PGWR was seen with a pressure of 50 cm H_2O. Both serum and more noticeably peritoneal fluid amylase levels were elevated. Histological assessment of the pancreas revealed evidence of mild to moderate pancreatic damage, with little increase in the damage with increased pressure. The changes seen were those of marked inflammatory infiltrate, moderate oedema, acinar necrosis and duct inflammation. There was little evidence of haemorrhage and no animal developed acute haemorrhagic pancreatitis. The microscopic appearance of pancreatic damage was completely different to that seen with trypsin or enterokinase. Whilst trypsin and enterokinase produced predominantly oedema with minimal inflammation, GDC gave duct inflammation, acinar necrosis and a marked inflammatory infiltrate.

Enterokinase and glycodeoxycholate

Immediately on injection of GDC and enterokinase moderate oedema was produced. No animal died or developed acute haemorrhagic pancreatitis within the 24 hour period. At sacrifice the macroscopic appearances were those of moderate pancreatitis with marked oedema, ascites and fat necroses and these appearances were far worse than those seen after enterokinase alone and more severe than after GDC alone. When the markers of pancreatic damage were assessed fully these differences became less marked as only the serum amylase showing an increase over GDC infusion alone. Histology after 50 cm H_2O pressure infusion showed moderate pancreatic damage with microscopic evidence of inflammatory cell infiltrate, oedema, duct inflammation and acinar necrosis. These changes were very similar in character and magnitude to those seen with GDC infusion alone indicating that the addition of low dose enterokinase did not appreciably increase the toxic potential of glycodeoxycholate.

Trypsin and glycodeoxycholate

Severe oedema was noticed immediately after infusion of 50 µl of this solution at all pressures. Again all animals survived to 24 hours and none developed acute haemorrhagic pancreatitis. At sacrifice there was moderate pancreatitis with marked oedema, fat necrosis and ascites. There was no consistent difference in the pancreatic damage observed compared to either GDC or trypsin given individually with only moderate elevations in serum and peritoneal fluid amylase. The histological changes were those of oedema, inflammatory infiltrate, acinar necrosis and duct inflammation. i.e. a combination of enterokinase and trypsin effects. Therefore when trypsin and GDC were infused in combination the resultant pancreatic damage was not increased but was rather a mixture of the two effects.

Trypsin and enterokinase and glycodeoxycholate

This mixture produced severe damage to the pancreas and several animals developed acute haemorrhagic pancreatitis. No animal died before sacrifice although several rats appeared to be in a poor clinical state. Two to three minutes after infusion the gland became intensely oedematous with haemorrhages and evidence of red and swollen ducts. At sacrifice there was marked pancreatic damage with multiple fat necroses, severe ascites, gross oedema and haemorrhages. The appearances were those of moderate to severe pancreatic damage. The PGWR was significantly elevated ($P<0.01$) at all pressures and both serum ($P<0.02$) and pancreatic fluid ($P<0.01$) amylase levels were greater than those obtained after the previous infusions. The histological features were those of marked oedema, acinar necrosis, inflammatory infiltrate, duct damage and haemorrhage. The histological score obtained was twice as high as with the other infusions at all

pressure ($P < 0.001$). Acute haemorrhagic pancreatitis or severe pancreatic damage is compared with pressure in this table.

pressure (cm H_2O)	percentage of animals developing haemorrhagic pancreatitis
15	10
20	20
25	60
50.	80

Infusion of a mixture of glycodeoxycholate, trypsin and enterokinase produced severe damage to the pancreas. Although this damage was most severe at high pressure there was a significant number of animals who developed haemorrhagic pancreatitis at moderate and even low pressures.

Results summary (table 16).

1. infusion of trypsin and enterokinase produced pancreatic oedema.
2. bile salt (GDC) infusion gave duct inflammation, acinar necrosis and an inflammatory infiltrate.
3. combinations of GDC with enterokinase or trypsin produced a mixture of effects without, however, a marked increase in pancreatic damage.
4. a mixture of GDC/enterokinase/trypsin was toxic to the pancreas and many animals developed haemorrhagic pancreatitis.
5. these results were dependent on pressure with maximum damage resultant on high pressure infusion (known duct rupture).

TABLE 12 PGW Ratio (g/100g)

PRESSURE (cm H_2O)

	No.	15	20	25	50
Saline	10	*324±30*	*341±35*	*361±26*	*408±24*
300u Trypsin	8	*406±13**	*410±26**	*421±17**	*450±31**
200ng Enterokinase	8	*400±21**	*389±26**	*394±23**	*424±16+*
200µg Enterokinase	8	*435±18**	*434±14**	*431±21**	*458±24**
10mM GDC	8	*412±14**	*430±8 **	*472±17**	*489±25**
200ng EK + GDC (10mM)	8	*440±18**	*414±23*	*432±27*	*493±31*
300u Trypsin + GDC(10mM)	8	*418±26*	*438±16*	*451±21*	*487±29*
Trypsin + EK + GDC 300u 200ng 10mM	8	*465±27**	*481±30**	*505±31**	*572±19**

Trypsin v. saline *P<0.001

EK (low) v. saline *P<0.01 +P<0.02

EK (high) v. saline *P<0.001

GDC v. saline *P<0.001

EK/GDC v. GDC *P<0.02, rest N.S.

Tryp/GDC v. GDC or Trypsin N.S.

Tr/EK/GDC v. all *P<0.01

TABLE 13 Serum Amylase (u/l)

PRESSURE (cm H_2O)

	No.	15	20	25	50
Saline	10	7225±2705	6467±3045	6373±2555	10295±1443
300u Trypsin	8	7800±2300	8400±2060	9100±3040	10900±480
200ng Enterokinase	8	6050±1800	8100±1760	9040±2040	10400±1560
200µg Enterokinase	8	10460±1780+	14700±4300*	23610±4080*	20280±3600*
10mM GDC	8	8670±865	8300±1600 *	9400±630*	12560±1410
200ng EK + GDC (10mM)	8	7516±1610	11205±1800*	12400±3040*	18900±2060*
300u Trypsin + GDC (10mM)	8	10290±1680*	10320±1410	14000±1890*	13600±2700
Trypsin + EK + GDC 300u 200ng 10mM	8	14100±2900*	18900±640*	22000±3000*	24200±4050*

Trypsin v. saline
N.S.

EK (low) v. saline
N.S.

EK (high) v. saline
+P<0.02, *P<0.01

GDC v. saline
*P<0.02

EK/GDC v. GDC
*P<0.02

Tryp/GDC v. GDC or Tryp.
*P<0.02

Tr/EK/GDC v. all
*P<0.02

TABLE 14 Peritoneal fluid Amylase (u/l x 10^3)

PRESSURE (cm H_2O)

	No.	15	20	25	50
Saline	10	*8.1±2.8*	*9.4±3.2*	*9.4±3.3*	*32.4±6.2*
300u Trypsin	8	*7.3±3.6*	*19.2±3.6**	*25.2±10.1**	*36.3±5.1+*
200ng Enterokinase	8	*9.4±6.1*	*10.2±1.6*	*12.6±4.8*	*27.3±9.3*
200µg Enterokinase	8	*13.6±5.2**	*14.2±2.8**	*17.9±6.1**	*41.3±14.0**
10mM GDC	8	*12.3±3.2**	*18.1±4.6**	*21.2±6.0**	*43.1±6.3**
200ng EK + GDC (10mM)	8	*13.2±4.2*	*19.3±5.1*	*24.6±5.5*	*44.3±4.2*
300u Trypsin + GDC (10mM)	8	*18.5±7.1**	*20.4±6.1*	*27.5±6.1*	*46.3±4.6*
Trypsin + EK + GDC 300u 200ng 10mM	8	*51.3±18.3**	*43.1±14.2**	*45.2±16.0**	*86.2±22.5**

Trypsin v. saline
*P<0.01,
+P<0.02

EK (low) v. saline
N.S.

EK (high) v. saline
*P<0.01

GDC v. saline
*P<0.01

EK/GDC v. GDC
N.S.

Tryp/GDC v. GDC or Trypsin
*P<0.02

Tr/EK/GDC v. all
*P<0.01

TABLE 15 Histology Score (0-150)
(mean of 8 x 10)

PRESSURE (cm H_2O)

	No.	15	20	25	50
Saline	10	9	10	12	16
300u Trypsin	8	26*	37*	40*	42*
200ng Enterokinase	8	22*	21*	23*	24*
200µg Enterokinase	8	50*	52*	54*	55*
10mM GDC	8	46*	48*	50*	54*
200ng EK + GDC (10mM)	8	42	48	53	56
300u Trypsin + GDC (10mM)	8	39	45	53	54
Trypsin + EK + GDC 300u 200ng 10mM	8	10% 76*	20% 85*	60% 96*	80% 112*

Trypsin v. saline
*P<0.01

EK (low) v. saline
*P<0.02

EK (high) v. saline
*P<0.001

GDC v. saline
*P<0.001

EK/GDC v. GDC
N.S.

Tryp/GDC v. GDC or Trypsin
N.S.

Tr/EK/GDC v. all
*P<0.001

% AHP.

TABLE 16 The effects of trypsin/enterokinase and GDC on the pancreas

Infusate	Pancreatic damage low press.	high press.	Histological change
EK	±	+	oedema
Trypsin	±	+	oedema
GDC	+	+	inflammation ducts acinar necrosis
EK + GDC	+	+	oedema inflammation ducts acinar necrosis
Trypsin + GDC	+	+	oedema inflammation ducts acinar necrosis
EK + Trypsin + GDC	++	+++	acute haemorrhagic pancreatitis

Discussion

These experiments have vindicated the policy of only studying pancreatic infusates when given at physiological volume and pressure, and we believe that the pathological changes resultant on such experimental conditions may well have relevance in the pathogenesis of acute gallstone pancreatitis.

In this study trypsin infusion alone, at concentrations used by Anderson et al (1969), produced immediate oedema of the gland. At 24 hours the appearances were those of oedematous pancreatitis with the degree of damage proportional to the pressure of infusion. These results are in keeping with the earlier studies of Anderson (1962, 1969) who noted the rapid accumulation of oedema fluid after trypsin injection. The activity of trypsin used in this study produced moderate damage to the pancreas although the toxic effects of larger doses (Creutzfeldt 1970) were not studied. Active trypsin appears to produce oedematous pancreatic damage when given under these "physiological" conditions. There was little evidence of acute inflammation and no acinar necrosis, duct changes or haemorrhage. Trypsin escapes from the duct via intercellular clefts (moderate pressures) or duct ruptures (high pressures) into the interstitium where it has a limited pathological effect, possibly due to the presence of trypsin inhibitor.

Enterokinase has recently been the source of much discussion, especially by the researchers from St Georges Hospital, London (Grant 1983, 1981; Terry 1982, 1983). This study investigated the effects of both low dose (200 ng) and high dose (200 µg) enterokinase on the rat pancreas. The importance of having previously studied the effects of pressure and saline alone became obvious when the limited pathological changes of low dose

enterokinase were evaluated. Low dose enterokinase produced damage only marginally worse than that of saline alone with moderate oedema at the higher infusion pressures. In contrast high dose enterokinase produced severe oedema with a mild inflammatory infiltrate - changes corresponding to those of trypsin infusion. No animal developed haemorrhagic pancreatitis - results similar to those of Terry (1982). Earlier experiments using enterokinase infusion have produced acute pancreatitis (Mann 1979, Hammond 1977, Mann 1969, 1977), but these studies are not comparable as large volumes of concentrated enterokinase were given at high pressure. Enterokinase produces oedematous pancreatic damage only, with the changes produced being related to the pressure of injection and concentration of enterokinase.

Glycodeoxycholate (GDC) was chosen for a study of the effects of bile salts on the pancreas for several reasons: (a) in man the glycine:taurine ratio is 3:1, (b) deoxycholate is the most active bile salt, and (c) patients with gallstones have elevated deoxycholate levels in bile. The bile salt concentration of 10 mM used in this study is similar to that seen in the common bile duct of man (Reber 1979, Carey 1970) and is less than the 34 mM concentration used by Terry and associates (1982, 1983). Injection of GDC into the pancreatic duct produced little immediate change as opposed to the immediate oedema after trypsin and enterokinase. Moderate pancreatic damage was observed at 24 hours which was of a different nature to that seen after trypsin and enterokinase. GDC produced duct damage, acinar necrosis and an inflammatory infiltrate, changes suggestive of a detergent action on cell membranes. These changes re-emphasize Beck's (1971) observations of the two types of pancreatic damage i.e. the production of oedema by trypsin and coagulation necrosis

by bile. Importantly no animal developed acute haemorrhagic pancreatitis as a result of GDC infusion alone. The changes produced by GDC were exacerbated by pressure, an observation later discussed in detail in connection with the "pancreatic duct mucosal barrier".

A combination of GDC and enterokinase produced moderate pancreatic damage, little worse than after bile salt alone. These results, although not strictly comparable with those of Terry (1982, 1983), demonstrated no obvious increase in the pathogenicity of bile salts when active enterokinase was added. Similarly a mixture of trypsin and GDC produced moderate pancreatic damage, the histological appearances being those of trypsin and bile salts alone. Elmslie's (1963, 1966) observations appear to be correct i.e. a fresh mixture of bile salt and trypsin, given at physiological volumes and pressures, is no more pathogenic than bile salt alone.

Infusion of a trypsin, enterokinase and bile salt mixture caused a marked increase in pancreatic damage. This mixture resembles the fluid found in closed loop experimental preparations (Orda 1980, Pfeffer 1957, Nevalainen 1975, Durst 1971, Rao 1981, Chetty 1980, Ferrie 1978), which consistently produce acute haemorrhagic pancreatitis. Infusion of this mixture of substances, at presumptive physiological concentrations, volumes and pressures, produced severe acute pancreatitis with many animals having the acute haemorrhagic form. Duct extravasation of bile salts, active trypsin and enterokinase might initiate the cascade of enzymes (Creutzfeldt 1970, Durr 1979) and overcome the protective action of trypsin inhibitor. This hypothesis was evidenced by the increased incidence of haemorrhagic pancreatitis with pressure as higher pressure

with its attendent greater extravasation of duct contents was associated with more severe pancreatic damage.

This study has demonstrated the difficulty in producing severe pancreatitis when all variables are controlled within the physiological range. Bile salts, trypsin and enterokinase given alone produce only mild to moderate pancreatitis and only when a mixture of all three is infused do animals develop acute haemorrhagic pancreatitis. The results obtained have emphasized the role of pressure: at low pressures (15 cm H_2O) only mild damage occurs; at moderate pressures (20, 25 cm H_2O) with leakage through intercellular clefts moderate damage results and at high pressure (50 cm H_2O) with known duct rupture marked toxicity ensues. The effects of pressure, previously studied with an inert saline solution, are more pronounced when the duct contents are toxic. Although it might be argued that our occlusion time of 5 minutes was too short, we are of the opinion, in the context of gallstone migration, that this time is more representative than total occlusion for 24 hours.

Conclusions

(1) Trypsin and enterokinase alone produced oedematous pancreatitis.

(2) Bile salts gave moderate pancreatic damage.

(3) A combination of glycodeoxycholate, enterokinase and trypsin was extremely toxic to the pancreas.

(4) All the above effects were closely related to pressure. High pressure was accompanied by more severe changes.

CHAPTER VII

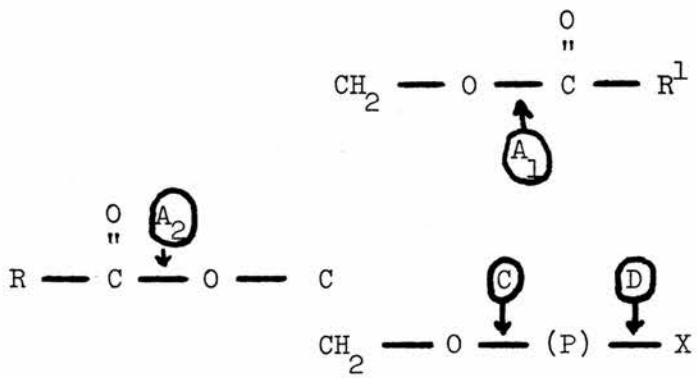
PHOSPHOLIPASE A_2 AND LYSOLECITHIN AND THE PANCREAS

Introduction

For many years trypsin and the other pancreatic proteases were considered to be completely responsible for the auto-digestive changes in acute pancreatitis. Recently, however, there has been considerable interest concerning the possible role of pancreatic phospholipase A_2 in both the initiation and autodigestion of acute pancreatitis.

Phospholipase A_2 (PLA_2):

Phospholipases are hydrolytic enzymes of quite widespread occurrence in nature (Nevalainen 1980). Four types of phospholipase have been described by virtue of their site of action on a phospholipid molecule i.e., A_1, A_2, C, D.



- R R^1 - fatty acids
- X e.g. choline

(Blackwell 1983).

Phospholipid

Phospholipases C and D are mostly found in bacteria. PLA_1 is labile to heat, whereas PLA_2 is stable to heat. Both PLA_1 and PLA_2 exist either membrane bound (active at neutral pH and require Ca^{2+} for action) or in lysosomes (active at acidic pH, don't require Ca^{2+}) (Blackwell 1983, Nevalainen 1980, de Haas 1965). Phospholipase A_2 is the enzyme most

frequently studied of the two (PLA_1 and A_2) in connection with acute pancreatitis, since it is easily separable from other pancreatic enzymes owing to its heat stability (Nevalainen 1980). Phospholipase A_2 is present in pancreatic tissue and juice almost exclusively as an enzymatically inactive precursor (Arnesjo 1967, Nevalainen 1980, Creutzfeldt 1970, Aho 1982). This pro-phospholipase A_2 is converted into active PLA_2 by trypsin with the splitting of 7 amino acids from the zymogen molecule (Wanke 1970). PLA_2 consists of 123 amino acids with a molecular weight of 13,900.

The richest sources of PLA_2 are snake venom (cobra), bee venom and mammalian pancreatic tissue - man, ox, rat, horse, pig, guinea pig and sheep. Compared with the animal species that have been investigated, the human pancreas contains large amounts of this enzyme (Zieve 1963, Creutzfeldt 1970, Nevalainen 1980) with 10 to 20 times higher concentrations. There are several specific requirements for the enzymatic activity of PLA_2. Pancreatic PLA_2 is inactive in the absence of bile salt and Ca^{2+} and is optimally active at pH 8.5 to 9.0 (Nevalainen 1980). PLA_2 is synthesized as an inactive precursor by pancreatic acinar cells, liberated to pancreatic juice after stimulation of the gland and secreted for digestive purposes into the duodenum. Activation occurs in the duodenum. Its main action is on phospholipid thus:

$$\text{phospholipid} \xrightarrow{PLA_2} \text{Lysophospholipid} + \text{Fatty acid.}$$

As phospholipids are the main lipid constituents of cellular membranes (Nevalainen 1980) active PLA_2 has a direct cytotoxic action.

The intraductal instillation of PLA_2 alone does not induce pancreatic damage and the damage only becomes significant if small amounts of bile

salts are present (Nevalainen 1980, Wanke 1970, Schmidt 1969). Furthermore, whilst PLA_2 given at high pressure will induce severe pancreatic damage, the same PLA_2 at low pressure produces only negligible pancreatic toxicity (Banks 1970, Anderson 1969). Anderson and colleagues (1969) investigated the effects of PLA_2 on the canine pancreas. After high pressure infusion of a large concentration of the enzyme they demonstrated immediate changes in the gland with inevitable acute haemorrhagic pancreatitis. These changes were much more severe than those seen after trypsin or chymotrypsin infusion.

Aho and Nevalainen (1982) carefully studied the early pancreatic lesions induced by murine intraductal injection of PLA_2 with bile salts. The histological changes were those of early (15 minutes) necrosis of acinar cells and later (3 hours) vacuolation of the cytoplasm. On electron microscopy early injury of cells was demonstrated with vesiculation of the endoplasmic reticulum - at 3 hours the cells were extensively damaged. The most significant change observed after PLA_2 infusion was widespread acinar necrosis. Indeed Aho (1982, 1980c) postulated that PLA_2 was perhaps the key enzyme in acute pancreatitis. This postulate becomes more probable in the light of the finding of PLA_2 in pancreatic tissue, peritoneal fluid and peripheral blood of dogs with acute pancreatitis (Nevalainen 1980). Moreover, PLA_2 has now been demonstrated in the peritoneal fluid and serum of patients with acute pancreatitis (Tykka 1980).

PLA_2 can be inhibited by several substances e.g., EDTA, zinc, lead, antimalarials, several antibiotics and local anaesthetics (Tykka 1980). Use has recently been made of these observations in the treatment of acute pancreatitis with promising results (Tykka 1980).

Pancreatic PLA_2 is found in large amount in the human pancreas. Once activated it may damage the pancreas either through a direct attack on membrane phospholipids or through formation of lysolecithin from lecithin in bile (Boyle 1984).

Lysolecithin

Phospholipids are second only to bile salts as a major component of bile, having a concentration of 1 to 6 mg/ml in hepatic bile and 10 to 58 mg/ml in concentrated gallbladder bile (Haslewood 1968, Poncelet 1972, Martin 1981). In man 95% of the phospholipids are lecithins (phosphatidyl choline), primarily palmitoyl - β - oleyl-lecithin (Poncelet 1972). Active PLA_2 in the presence of bile salts and Ca^{2+} ions selectively removes the β 1 fatty acid from the lecithin molecule to give lysolecithin (Nevalainen 1980). This reaction is very rapid and occurs normally in the duodenum with 100% of the bile lecithins being converted to lysolecithins. It could also occur in the biliary tree (Poncelet 1972). In vitro bile lecithins are completely converted to lysolecithins by pancreatic juice in a period of three to four hours (Schmidt 1969). Lysolecithins as a group are very toxic to cellular membranes and their toxicity is directly proportional to their saturation and the chain length of their constituent fatty acid (Poncelet 1972). Most studies with lysolecithins have been concerned with their action on red blood cell membranes, to which they are extremely damaging (Boyle 1984, Poncelet 1972, Reeman 1967), and it is well established that lysolecithins are extremely cytotoxic.

Arnesjo (1971) studied the formation of lysolecithin in rat pancreas following experimentally induced bile salt pancreatitis. He found

that following injection there was a progressive conversion of rat pancreatic lecithin to lysolecithin - more marked in necrotic areas of the gland. In contrast however, Papp and co-workers (1973) could find no increase in lysolecithin after canine pancreatic duct injection of bile salt. Their results cast considerable doubt on the validity of the assumption that lysolecithin is responsible for pancreatic injury during the early phases of acute experimental pancreatitis. Notwithstanding this finding there is now a weight of evidence which implicates lysolecithin in the early stages of acute pancreatitis (Nevalainen 1980, Aho 1982).

Reflux of bile or duodenal juice into the pancreatic duct provides a substrate (lecithin) for lysolecithin formation by active PLA_2. Poncelet and Thompson (1972), from Montreal, studied the action of bile phospholipids on the pancreas. They injected lysolecithin into the rat pancreas and evaluated pancreatic toxicity at one to four days. In their study lysolecithin produced pancreatic necrosis and interstitial bleeding at concentrations of 6 mg/ml and above. They concluded that lysolecithin was very toxic to the rat pancreas when infused at concentrations well within the levels of lysolecithin found in human bile. Unfortunately, however, their study used a 1000 µl infusion at 25 cm H_2O, which we have previously shown to be associated with gross duct rupture, and therefore the pathophysiological relevance of their observations remains uncertain.

Recently Aho and Nevalainen (1982) induced lesions in the rat pancreas by injecting 200 µl of 2.5% (25 mg/ml) lysolecithin into the ducts. The changes observed occurred very quickly after injection (15 minutes) and were similar to those of PLA_2 infusion (see earlier). The most noticeable

change appeared to be that of acinar necrosis. Again this study can be criticized for using a supraphysiological volume with no pressure recordings.

Lysolecithin is a naturally occurring detergent which can break down mucus structures and is directly toxic to membranes (Martin 1978, Kellaway 1977). Indeed the presence of lysolecithin in the stomach has been implicated in the pathogenesis of peptic ulceration (Martin 1981, Johnson 1974, Boyle 1984, Lawson 1964, Silen 1976, Davenport 1970, Kivilaakso 1978, Duane 1980). These membrane or barrier breaking properties have been investigated at length later in this thesis and are described in Chapter XI.

Several conclusions can be drawn from these reports.

1. Phospholipase A_2 requires bile salts and Ca^{2+}, both found in bile, to be active.
2. Phospholipase A_2 acts on bile to produce lysolecithin.
3. Active phospholipase A_2 and lysolecithin are toxic to membranes and can cause acute experimental pancreatitis.
4. There is no evidence that either phospholipase A_2 or lysolecithin when given in physiological concentrations, volumes and pressures is pancreatotoxic.
5. Whilst the role of phospholipase A_2 and lysolecithin in the initiation of acute pancreatitis remains speculative, their part in the later autodigestive process appears well established.

Materials and Methods

Object

To investigate the effects of lysolecithin and phospholipase A_2 on the rat pancreas. The 50 µl volume was used as previously described and note was taken of the pressure of injection.

Experimental preparation

A 50 µl solution containing phospholipase A_2 or lysolecithin with or without bile salt was infused into the rat pancreas (fig. 5) at pressures of 15, 20, 25, 50 cm H_2O. Cannula occlusion was maintained for 5 minutes only. The animals made a full recovery until sacrifice at 24 hours. Groups of 8 animals were studied.

Solutions

Phospholipase A_2. (Sigma, P-9139 from porcine pancreas suspended in 3.2M $(NH_4)_2\ SO_4$ solution pH 5.5: 680 units/mg protein: 1 unit hydrolyzes 1.0 umol of L-α phosphatidyl choline to L-α lysophosphatidyl choline and a fatty acid per minute at pH 8.0 at 37°C). A fresh solution of phospholipase A_2 (PLA_2) was prepared by adding PLA_2 to sterile 0.9% saline to give a concentration of 25 u PLA_2/50 µl. This concentration is similar to that studied by Aho (1982).

Phospholipase A_2 and bile salt.

A fresh solution was prepared by adding glycodeoxycholic acid (Sigma, G-3258) to phospholipase A_2 in saline to give a final concentration of PLA_2 25 units and GDC 10mM in a 50 µl volume.

Lysolecithin. (Sigma, L-4129, L-α phosphatidyl choline, egg yolk - type I or Sigma L-5254, L-α phosphatidyl choline palmitoyl).

A fresh solution of lysolecithin was prepared by adding L-∝phosphatidyl choline to sterile 0.9% saline to give final concentrations of 1% (10 mg/ml) or 2.5% (25 mg/ml). As the two different lysolecithins used gave equivalent results, only the L-∝phosphatidyl choline was studied in depth. The concentrations of lysolecithin chosen were those known to occur in human bile (Poncelet 1972, Haverback 1960, Martin 1981, Aho 1982).

Lysolecithin and bile salt.

Glycodeoxycholic acid (Sigma, G-3258) was added to a 1% lysolecithin solution to give a final concentration of glycodeoxycholic acid 10 mM, lysolecithin 1%.

Results (summarized in Tables 17 to 20).

Phospholipase A_2.

Infusion of PLA_2 produced no immediate change in the pancreas. There was no significant difference in pancreatic damage from saline only, as assessed by PGWR, serum and peritoneal fluid amylase levels, and histology score at 24 hours. At sacrifice the glands showed oedema only. The only histological abnormality was that of pressure related oedema as reported earlier after saline infusion.

Phospholipase A_2 and bile salt.

Infusion of a mixture of PLA_2 and GDC produced immediate oedema in the pancreas at all pressures. No animal died within the 24 hour period. At sacrifice pancreatic damage was significantly ($P<0.01$) worse by all 4 parameters than saline infusion alone at all pressures. The macroscopic appearance was that of moderate pancreatitis with oedema, fat necrosis, ascites with a few small haemorrhages. Pancreatic damage assessed by PGWR and amylase levels, correlated with pressure with maximum changes observed at 50 cm H_2O. A comparison of the pancreatic damage with that described (see previous Chapter) after GDC alone revealed only a subtle increase in pancreatic damage - both PGWR ($P<0.02$) and serum amylase ($P<0.01$) were increased but not peritoneal fluid amylase or the histology score. The histological changes observed after PLA_2/GDC infusion were not related to pressure as at low, medium and high pressures the appearances were similar. The microscopic features noted were those of marked acinar necrosis and moderate oedema, duct changes and inflammatory infiltrate. No animal developed acute haemorrhagic pancreatitis. Indeed analysis of the histology score revealed mild to moderate pancreatic

damage only.

Thus, addition of phospholipase A_2 to bile salt, whilst not appreciably altering the degree of pancreatic damage, does change the histological picture to one of widespread acinar necrosis.

Lysolecithin.

Pancreatic infusion of lysolecithin produced immediate oedema at pressures above 20 cm H_2O. Pancreatic damage at all pressures was markedly worse than after corresponding saline infusions; PGWR ($P < 0.001$), SA ($P < 0.02$), PFA ($P < 0.01$) and histology score ($P < 0.01$). A comparison of the two concentrations (1% and 2.5%) of lysolecithin revealed that a 2.5% solution produced more marked pancreatic damage, especially noticeable at the higher pressures. At 24 hours all animals were alive although several who had received 2.5% lysolecithin were in a poor clinical condition. At sacrifice the macroscopic appearance in most animals was that of moderate pancreatitis with oedema, fat necroses and ascites. Three animals had evidence of haemorrhage into the gland and these had received a 2.5% solution at 50 cm H_2O pressure. The histological features observed were more apparent in the glands of animals who had received a 2.5% lysolecithin infusion. The microscopic appearance of the glands was that of marked acinar necrosis, moderate oedema and inflammatory infiltrate and mild duct changes and haemorrhage. One animal, who had received a 2.5% infusion at 50 cm H_2O was classified as having histological severe or acute haemorrhagic pancreatitis.

Lysolecithin in presumptive pathophysiological concentrations was toxic to the pancreas at all pressures of injection. The major changes seen

in the pancreas were those of acinar necrosis and inflammatory infiltrate.

Lysolecithin and bile salt.

Injection of a mixture of lysolecithin and glycodeoxycholate produced immediate oedema with a few small haemorrhages in the pancreas. No animal died within the 24 hour period although the clinical state of a number of animals was poor at sacrifice. At sacrifice the macroscopic appearance of the gland was that of moderate to severe pancreatic inflammation (the most severe changes seen in the pancreases of animals who had received an infusion at 50 cm H_2O pressure), with oedema, fat necroses, peritoneal fluid and haemorrhage. In tables 17 to 20 the damage produced by a lysolecithin/GDC mixture has been compared to that of equivalent concentrations of GDC or lysolecithin alone. The PGWR was significantly higher in those animals receiving lysolecithin/GDC than those receiving GDC ($P < 0.01$) or lysolecithin ($P < 0.05$) alone. At low pressures (15, 20 cm H_2O) the differences were maximal. Likewise both serum and peritoneal fluid amylase levels were higher in those animals who had received lysolecithin/GDC than GDC (SA: $P < 0.02$) or lysolecithin (SA: $P < 0.01$, PFA: $P < 0.01$) alone. Again these differences were maximal with low pressure infusion. The histological features of animals receiving a pancreatic infusion of lysolecithin/GDC mixture were those of severe acinar necrosis, moderate oedema and inflammatory infiltrate, mild duct changes with occasional haemorrhagic foci. Two animals developed acute haemorrhagic pancreatitis, one each at infusion pressures of 25 and 50 cm H_2O. A comparison of the histological score with either GDC or lysolecithin revealed that the changes were more severe than with either alone ($P < 0.01$, $P < 0.02$ respectively). There appeared to be little change in the histology score with pressure. A mixture of

lysolecithin/GDC produced changes of a similar degree to that seen with PLA_2/GDC together.

Therefore, in this study, a mixture of lysolecithin and bile salt at physiological concentrations was more toxic to the pancreas than either lysolecithin or bile salt alone. The main histological change was that of acinar necrosis and the changes were less dependent on pressure than previously studied compounds.

Summary of results

1. Phospholipase A_2 alone was not toxic to the pancreas. When combined with bile salt, the active mixture produced moderate pancreatic damage.
2. Lysolecithin was toxic to the pancreas and this toxicity was increased when lysolecithin was combined with bile salt.
3. Pressure was less important in determining the degree of pancreatic damage when lysolecithin or phospholipase A_2 was infused.
4. The main histological feature produced by phospholipase A_2 and lysolecithin was acinar necrosis.
5. At these volumes and pressures acute haemorrhagic pancreatitis can result on infusion of physiological concentrations of lysolecithin.

TABLE 17 PGW Ratio (g/100g)
N = 8, each group

PRESSURE ($cm\ H_2O$)

	15	20	25	50
Saline	324±30	341±35	361±26	408±24
25u Phospholipase A_2	326±16	331±16	364±24	385±21
GDC (10mM)	412±14	430±8	472±17	489±25
25u P'LA_2 + GDC (10mM)	388±18*	465±18*+	504±21*+	524±16*+
Lysolecithin 1%	405±41*	423±18*	465±34*	474±21*
Lysolecithin 2.5%	434±16*+	478±21*+	482±16*‡	489±14*‡
1% Lysolecithin + GDC (10mM)	476±24*+	460±18*+	483±14‡	492±16‡

PLA_2 v. saline N.S.

PLA_2/Bile salt v. saline *P< 0.001

PLA_2/Bile salt v. GDC +P< 0.02

Lysolecithin v. saline *P< 0.001

Lyso. 2.5% v. 1% +P<0.02, ‡P<0.05

1% Lyso/GDC v. GDC *P<0.01

1% Lyso/GDC v. 1% Lysolecithin +P<0.01, ‡P<0.05

TABLE 18 Serum Amylase (u/l)
N = 8, each group

PRESSURE (cm H_2O)

	15	20	25	50
Saline	7225±2705	6467±3045	6373±2555	10295±1443
25u Phospholipase A_2	6100±460	5900±1460	6200±1240	8900±2040
GDC (10mM)	8670±865	8300±1600	9400±630	12560±1410
25u P'LA_2 + GDC (10mM)	12510±1450*+	12000±2040*+	14700±1680*+	19800±6060*+
Lysolecithin 1%	9100±1460*	9500±640*	10900±1400*	11200±1460
Lysolecithin 2.5%	10300±1520*	8600±1410*	12500±1640*	14500±960*+
1% Lysolecithin + GDC (10mM)	15310±1950*+	12800±4020*+	18900±3060*+	22500±1450*+

PLA_2 v. saline N.S.

PLA_2/Bile salt v. saline *P< 0.01

PLA_2/Bile salt v. GDC +P< 0.01

Lysolecithin v. saline *P< 0.02

Lyso. 2.5% v. 1% +P< 0.05

1% Lyso/GDC v. GDC *P< 0.01

1% Lyso/GDC v. 1% Lysolecithin +P< 0.01

TABLE 19 Peritoneal fluid amylase (u/l x 10^3)
(N = 8, each group)

	PRESSURE (cm H_2O)			
	15	20	25	50
Saline	8.1±2.8	9.4±3.2	9.4±3.3	32.4±6.2
25u Phospholipase A_2	6.9±1.8	9.2±4.6	8.3±6.1	28.6±4.2
GDC (10mM)	12.3±3.2	18.1±4.6	21.2±6.0	43.1±6.3
25u P'LA_2 + GDC (10mM)	16.4±4.6*	18.6±5.2*	26.3±4.2*	40.3±5.2*
Lysolecithin 1%	16.2±4.0*	19.3±5.1*	20.3±5.1*	40.3±5.1*
Lysolecithin 2.5%	18.3±6.3*	26.1±4.6*+	25.2±5.0*+	44.5±3.6*‡
1% Lysolecithin + GDC (10mM)	29.6±7.2*‡	41.3±6.1*‡	40.5±7.2*‡	49.2±8.1*‡

PLA_2 v. saline N.S.

PLA_2/Bile salt v. saline *P< 0.01

PLA_2/Bile salt v. GDC N.S.

Lysolecithin v. saline *P< 0.01

Lyso. 2.5% v. 1% +P<0.02, ‡P<0.05

1% Lyso/GDC v. GDC *P<0.001, +P<0.02

1% Lyso/GDC v. 1% Lysolecithin ‡P<0.01

TABLE 20 Histology Score (0-150)
(mean of 8 x 10) (N = 8, each group)

	PRESSURE (cm H_2O)			
	15	20	25	50
Saline	*9*	*10*	*12*	*16*
25u Phospholipase A_2	*10*	*12*	*13*	*15*
GDC (10mM)	*46*	*48*	*50*	*54*
25u P'LA_2 = GDC (10mM)	*45**	*45**	*49**	*50**
Lysolecithin 1%	*62**	*66**	*69**	*71**
Lysolecithin 2.5%	*69**	*73**	*79*+*	10% *83*+*
1% Lysolecithin + GDC (10mM)	*75*+*	*76*+*	10% *82*+*	10% *85*+*

PLA_2 v. saline N.S.

PLA_2/Bile salt v. saline *P< 0.001

PLA_2/Bile salt v. GDC N.S.

Lysolecithin v. saline * P< 0.01

Lyso. 2.5% v. 1% +P< 0.02

1% Lyso/GDC v. GDC *P< 0.01

1% Lyso/GDC v. 1% Lysolecithin +P< 0.02

% AHP

Discussion

This study has been the first to evaluate the toxic potential to the pancreas of lysolecithin and phospholipase A_2 (PLA_2) when given at presumptive physiological concentrations, volumes and pressures. Indeed, all of the earlier work concerning the role of PLA_2 and lysolecithin in the pathogenesis of acute pancreatitis has used volumes of fluid which we have shown to consistently produce ductal rupture. As reflux of these volumes is not possible in the clinical setting we have used a 50 µl volume throughout these experiments. In addition, careful consideration was given to the pressure of injection as this could well be relevant in the light of the previously described ductal extravasation.

In considering bile or duodenal reflux as likely initiators of acute gallstone pancreatitis both phospholipase A_2 and lysolecithin have potential importance. Bile contains large quantities of lecithin (Poncelet 1972, Nevalainen 1980), the natural substrate for PLA_2. When bile refluxes into the pancreatic duct the enzyme PLA_2 becomes active after contact with bile salts and Ca^{2+} and acts on lecithin to form lysolecithin (Schmidt 1969). In the presence of infected bile virtually all the lecithin is present in the lyso - form (Poncelet 1972), an observation which might be an important cause of the previously demonstrated toxicity of infected bile. In the duodenum, PLA_2 acts on the lecithin of bile to produce lysolecithin. This lysolecithin is not toxic to the duodenum but when refluxed into the pancreatic duct it can exert its toxic effects. PLA_2 is an enzyme with a pH optimum of 8.5-9.0, values which are normally found in bile and duodenal juice. Thus the enzyme PLA_2 and its toxic product lysolecithin could cause pancreatic damage through either duodenal

or bile reflux.

In this study pure PLA_2 (at a concentration of 25 u/50 µl) was non toxic to the pancreas, confirming the earlier observations of Schmidt (1967), Wanke (1970) and Arnesjö (1971). In vitro PLA_2 is inactive in the absence of bile salt and calcium when lecithin is used as the substrate (Arnesjö 1970). PLA_2 solution produced changes in the pancreas indistinguishable from those of corresponding infusions of saline. We were unable to confirm the findings of Aho (1982) who found acinar necrosis after infusion of pure PLA_2 (the same enzyme as in this study). However, the experiments are not strictly comparable since he was using 200 µl of enzyme without attention given to pressure.

When PLA_2 and glycodeoxycholic acid (GDC) were infused together much more severe damage occurred than after PLA_2 alone. A comparison with the known pancreatic toxicity of GDC alone revealed only a slight increase in pancreatic damage with the PLA_2/GDC mixture. However, when PLA_2 was added to GDC the histological picture was very different - acinar necrosis became marked. These histological changes did not appear to be related to infusion pressure, indicating that the PLA_2/GDC mixture was toxic to the "pancreatic duct mucosal barrier" (Reber 1979). The findings of marked acinar necrosis when active PLA_2 was infused into the pancreas are similar to those observed by Aho (1982). This report suggests that PLA_2 and GDC leave the duct, either by pressure induced extravasation or by direct toxicity to the duct integrity, and pass into the interstitium where active PLA_2 is free to exert its toxic effect on acinar cells.

There are several chemical types of lysolecithin which differ in their

fatty acid chains. This study investigated the effects of L-phosphatidyl choline and its palmitoyl conjugate and found their pancreatotoxicity to be very similar. This is in keeping with their comparable degree of saturation and chain length of the constituent fatty acid (Reeman 1967, Poncelet 1972). The concentrations of lysolecithin studied (1% and 2.5%) were well within those known to occur in the human biliary tree or duodenum (Boyle 1984, Poncelet 1972, Aho 1982, Nevalainen 1980). At these concentrations lysolecithin has been shown to damage gastric mucosa and be possibly responsible for gastric ulceration (Boyle 1984, Davenport 1970, Silen 1981, Martin 1981). To date, however, the direct toxicity of lysolecithin to the pancreas under physiological conditions remains uncertain. This study investigated the toxicity of lysolecithin on the pancreas as a whole and in chapter XI its effect on the "pancreatic duct mucosal barrier" is evaluated. Lysolecithin was responsible for moderate pancreatic damage with more severe changes noticed after infusion of the 2.5% solution. This toxicity was apparent at all pressures and suggests a direct effect on membrane permeability. The microscopic appearance in the gland was that of marked acinar necrosis and acute inflammatory infiltrate as previously described by Aho (1982) and in addition, one animal developed acute haemorrhagic pancreatitis. Therefore lysolecithin, at physiological concentrations, volume and pressures was toxic to the pancreas.

Both lysolecithin and bile salt are strong naturally occurring detergents (Helenius 1975) that have direct membrane toxicity. A mixture of these two compounds would therefore be expected to have marked toxicity to the pancreas. Indeed this was the case after infusion of a mixture of 1% lysolecithin and 10 mM GDC, concentrations well within the range found in bile

and duodenal fluid. Although no animals died within the 24 hour period the poor clinical state of several indicated severe acute pancreatitis. At sacrifice there was indeed macroscopic evidence of acute pancreatic inflammation with several glands showing acute haemorrhagic changes. When the markers of pancreatic damage were compared with those after bile salt or lysolecithin infusion alone several interesting features became apparent. There were significant elevations in PGWR, amylase levels and the histology score and these differences were maximal at low pressures. It appeared that a GDC/lysolecithin mixture was almost independent of pressure for its toxic effect. This independence from pressure was previously demonstrated by infected bile and to a lesser extent "pancreatitic" bile, lysolecithin alone and a PLA_2/GDC mixture. The greater detergent activity of these infusates could be the reason for this observation. Microscopic examination of the pancreas after lysolecithin/GDC infusion revealed moderate to severe pancreatic inflammation with marked acinar necrosis, oedema and inflammatory infiltrate. Acute haemorrhagic pancreatitis was observed in two of the 32 animals studied (6.2%). Thus the toxicity of lysolecithin was increased when in combination with bile salt. This observation might have relevance in the context of duodenal or biliary reflux into the pancreatic duct.

Conclusions

1. Active phospholipase A_2 produced moderate pancreatic damage.
2. Lysolecithin was toxic to the pancreas. When combined with bile salts there was a marked increase in toxicity.
3. Both phospholipase A_2 and lysolecithin produced severe acinar necrosis and this indicates a direct cytotoxic action.

4. As pressure is much less important in determining pancreatic damage, these natural detergents might well act by altering duct permeability (see later).

5. Lysolecithin can produce acute haemorrhagic pancreatitis under physiological conditions.

PART II

THE PANCREATIC DUCT

MUCOSAL BARRIER

CHAPTER VIII

THE PANCREATIC DUCT MUCOSAL BARRIER AND PANCREATIC DUCT INTEGRITY

Although the pathogenesis of acute gallstone pancreatitis continues to perplex present investigators, a weight of evidence now exists which indicates that the primary pathology may occur in the pancreatic ducts themselves (Steer 1984, Reber 1979a). Reflux of bile or duodenal contents into the pancreatic duct following passage of a gallstone through the ampulla of Vater is probably the originating mechanism. Following initial damage to the pancreatic duct, extravasation into the interstitium occurs and may progress to the enzymatic autodigestion so typical of acute pancreatitis. The amount of damage to the duct epithelium may be dependent on the following factors;

(i) constituents of bile,
(ii) constituents of duodenal juice,
(iii) the nature of pancreatic fluid,
(iv) the pressure within the duct,
(v) the amount of reflux,
(vi) the presence or absence of infection,
(vii) intrinsic defence factors within the duct itself.

The effect of these factors on duct integrity may hold the key to understanding the pathogenesis of gallstone pancreatitis. The concept of pancreatic duct integrity is new and is a logical development in thought from that of the "gastric mucosal barrier".

Gastric mucosal barrier

The inherent resistance of the gastric epithelium to autodigestion by acid and pepsin has puzzled physiologists for many years. Hunter (1772) killed a number of different animals at varying times after they had eaten and noted that, after death, the stomach wall was digested. He concluded that the dead stomach was no longer capable of resisting the digestive juice it had produced, whilst the living stomach had a barrier to protect itself from the effects of gastric acidity. Hollander (1954) proposed the concept of a gastric mucosal barrier to be composed of 2 integrated structural units, namely a layer of viscous mucus and a layer of tall columnar cells. He further conceived that the gastric mucosal barrier was not a static structure, but a truly dynamic system in which its two components were constantly being subjected to forces of destruction and replacement. The term "gastric mucosal barrier" is synonymous with the work of Davenport. He described a series of experiments in which he studied a variety of agents that altered the permeability or "damaged" the gastric mucosal barrier and thus allowed H^+ ions to diffuse rapidly from the gastric lumen (Davenport 1968, 1970a,b, 1972). Amongst the agents capable of damaging the barrier were short chain fatty acids, aspirin, ethanol and bile salts and it appears that damage to the barrier occurs within minutes of topical application of these substances (Morris 1984). Skillman (1970) further demonstrated that ischaemia or shock damaged the barrier.

The precise anatomical description of the gastric mucosal barrier remains elusive. Silen (1977) concluded that the gastric mucosal barrier is not one specific anatomical structure, but rather a dynamic ever-changing capacity of the stomach to withstand injury or ulceration. A consideration

of the factors involved in mucosal defence by the stomach is relevant in the context of the later discussion of the pancreatic duct. Surface protection of mucous membranes generally involves both acquired specific and innate non-specific defence factors, often functioning in intimate co-operation (Valnes 1984). Gastric epithelium is normally protected by mucin in combination with an efficient germicidal acid barrier. The surface epithelium is lined with a thin layer of a viscous mucus gel (Sarosiek 1984). The protective functions of this mucus depend strongly on the ability of its protein, glycoprotein, and lipid components to provide a highly hydrated mixing barrier capable of modulating the effects of various noxious agents. Damage to the gastric mucosal barrier is accompanied by solubilization and depletion of the mucus glycoproteins and glycolipids. Gastric mucosa also secretes HCO_3^- ions into the mucus, which may act as local buffering agents (Williams 1980). It has been further suggested that the columnar cells themselves or the tight junctions between cells represent the anatomical barrier (Davenport 1970b). Recently, Valnes (1984) has suggested that secretory immunoglobulin A antibodies and secreted lysozyme and lactoferrin, which have a broad spectrum of antibacterial properties, might be important in mucosal protection.

The gastric parietal cells secrete hydrogen ions in a concentration 10^6 times greater than that in blood and extracellular fluid. Despite this enormous concentration gradient, H^+ ions diffuse from gastric juice into the mucosa only slowly due to the "gastric mucosal barrier". Studies on impairment of this barrier have enabled a better understanding of the mechanisms of peptic ulceration.

Pancreatic duct mucosal barrier (PMB)

The pancreas secretes an alkaline juice with bicarbonate ions in concentrations five to six times higher than in blood. It has been suggested that the pancreatic duct may also possess a barrier to back diffusion, but in this case the contained ion is HCO_3^-. Secretin stimulation of the pancreas evokes the secretion of a fluid rich in Na^+ and HCO_3^-. This HCO_3^- containing fluid is secreted by cells in the fine ducts and acini of the pancreas and flows into the larger ducts. During maximal secretin stimulation, the HCO_3^- in the main pancreatic duct reaches 140-150 mM, while the HCO_3^- in the plasma is only 20-30 mM. The pancreatic juice, therefore, contains solute at concentrations greatly different from those in interstitial fluid and blood.

Several attempts have been made to investigate ionic movement across the main pancreatic duct by perfusing solutions of known composition along isolated segments. In the rabbit pancreatic duct Reber and collaborators (1969) found that at low flow rates a simulated pancreatic juice lost HCO_3^- and gained Cl^- whereas at high flow rates the concentration changed little. In the cat pancreas, Case et al (1969) found a similar loss of HCO_3^- and a gain of Cl^- when simulated pancreatic juice was perfused through the main duct. By application of Fick's law of diffusion they showed that this occurred by a passive process of exchange diffusion. Thus both Cl^- and HCO_3^- concentrations in the final pancreatic secretion are dependent on the rate of secretion:

At a low secretion rate pancreatic juice contains a high Cl^- and a low HCO_3^- concentration.

At a high secretion rate low Cl^- and high HCO_3^- concentrations are found.

In contrast the cations (K^+ and Na^+) remain at a constant concentration at all rates of secretion (Case 1979). Although ductal diffusion of anions permits a secondary modification of electrolyte composition, the duct HCO_3^- concentration is always higher and the Cl^- concentration lower than the corresponding blood levels. These observations prompted Reber (1979) to suggest that the pancreatic duct possessed a mucosal barrier that impaired HCO_3^- and Cl^- diffusion across the duct wall. This is shown diagrammatically in fig. 24. Moqtaderi and co-workers (1972) published data that supported the concept of a PMB. They found the rate of perfusion of the canine pancreatic duct related to HCO_3^- and Cl^- diffusion. When EDTA, a calcium chelating agent, was added there was a marked increase in anionic diffusion, implying that calcium was an integral part of the PMB stability.

Mosley and associates (1979) investigated transductal potential difference (pD) as a method of determining barrier integrity. The transductal pD in the cat pancreas (0 to -4.3 mV, lumen negative) was related to the difference in HCO_3^- between the perfusate and the blood. They felt that the pD represented a HCO_3^- concentration gradient across the duct wall.

The transductal potential difference of the pancreas is different to the pD used in assessing gastric mucosal barrier integrity, since the gastric mucosa is an actively secreting membrane and the resting stomach maintains a pD of -30 mV across the mucosa (Silen 1977). There is no evidence for active secretion by the main pancreatic duct and the pD across the resting duct is zero (Mosley 1979).

Like the gastric mucosal barrier, the precise anatomical location of the pancreatic duct mucosal barrier is not known. Greenwell (1977)

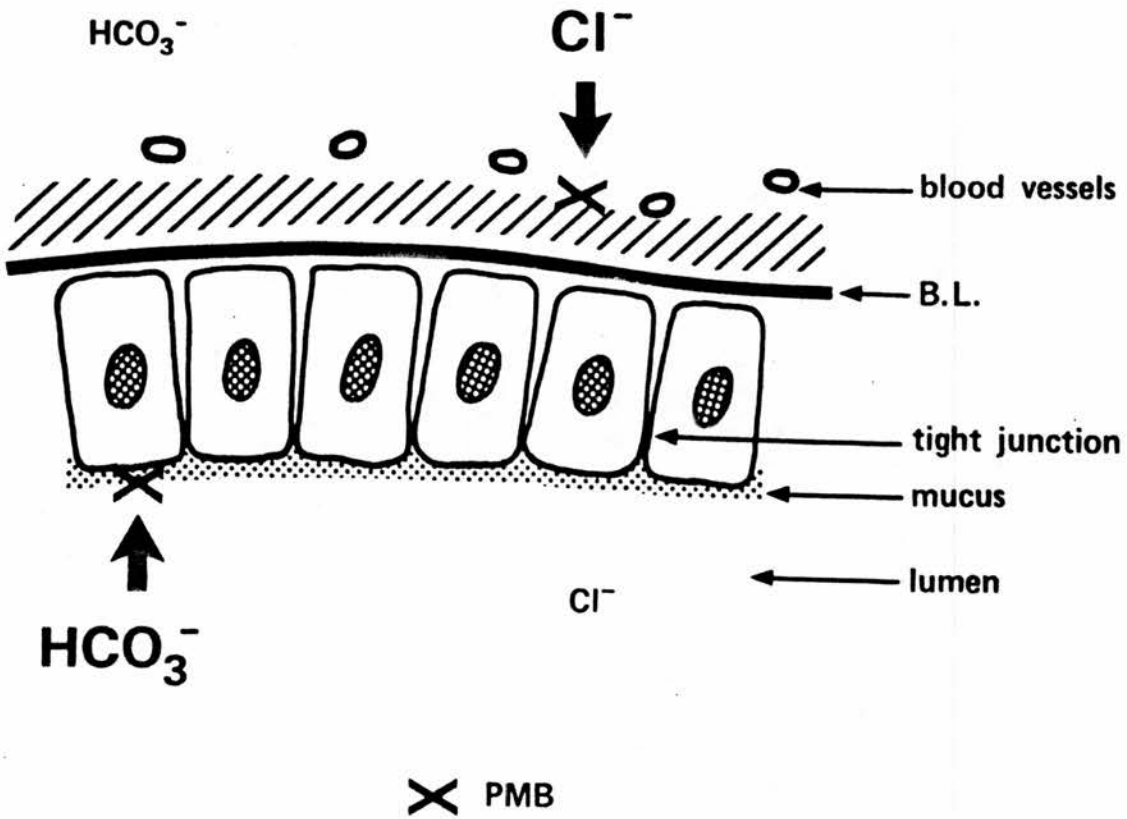


Fig. 24 Diagrammatic representation of pancreatic duct mucosal barrier (PMB)
BL = Basement Lamina
Heavy type = high [ionic] flux.

studied the permeability of the pancreatic duct to various ions and postulated that ion diffusion occurred at the tight junctions between cells rather than through epithelial cells. It appears that pancreatic duct epithelium is quite leaky (Case 1979). The paracellular pathway (quick movement) through junctional complexes, rather than the transcellular pathway (slow movement) is postulated to be the major route for passive ion permeation (Case 1979). The PMB is not an anatomical entity but rather a combination of factors that prevent free ionic movement across the duct wall. The duct wall itself has several important features: (i) mucus lining the cells, (ii) columnar cells, (iii) tight junctions, (iv) intercellular clefts, and (v) basement lamina, which may play a role in preserving duct integrity.

The importance of the mucus layer in maintaining pancreatic mucosal integrity has only recently been recognized. There are four types of mucus secreting epithelial cells in the pancreatic duct of man (Roberts 1972). These cells are located in different positions in the ductal system - types I, II, III in smaller ducts and types II, III, IV in larger ducts. The mucin of small ducts contains sulphated mucus and sialomucins and the mucin of larger ducts sialomucins and neutral glycoproteins. This change in mucins may reflect the ability of the ducts to resist digestion by pancreatic enzymes. Considering the presence of a barrier against back diffusion of bicarbonate ions and a possible protective effect of the mucus against pancreatic enzymes the mucins of the pancreatic duct may play a more important role than ever expected (Kodama 1983). Further evidence of the vital role of mucins in affording mucosal protection has been produced by Konok and Thompson

(1969) and Mizumoto (1971). They demonstrated that the pancreatic duct mucosal barrier was broken after the mucinous layer was destroyed by enzymes or infected bile.

The ultrastructure of the pancreatic duct is well described (Simpson 1983, Bub 1983, Reber 1981, Yoshizawa 1978, Kodama 1983). The ducts are lined by columnar cells with many short luminal microvilli (figs. 25A+B). These cells rest on a continuous basal lamina, supported in turn by delicate vascular connective tissue. Their cytoplasm contains mucous secretory granules, mitochondria, scanty endoplasmic reticulum and Golgi complex. The intercellular plasma membrane near the lumen has a tight junction and below this plasma membranes of adjacent cells are closely interdigitated. The apical junctional complex is illustrated in fig. 26 and the integrity of the surface epithelium may relate to the presence of these junctional complexes. Beneath the columnar cells is a continuous basal lamina which contains a unique collagen molecule (type IV) and two different glycoproteins of high and low molecular weight (Ham 1979). Analysis of the basement lamina reveals four basic components: (i) collagenous proteins, (ii) glycoproteins, (iii) proteoglycans, (iv) lipids. The function of the basement lamina is to provide an elastic support and possibly also to act as a filtration or diffusion barrier. The pancreatic duct mucosal barrier is, in our opinion, related to a combination of anatomical and physiological parameters. Damage to the barrier is associated with compromise to one or more of these parameters.

Most experimentation on ionic diffusion across the pancreatic duct walls has been performed in cats. These animals have several advantages as the basal pancreatic secretion is zero and the ducts themselves are

large enough to make surgical cannulation easy. Economic considerations are now a major concern of many laboratories, including our own, and thus there has been a search for cheaper animal preparations. Olazabal (1983, 1981) reported his experience with the rat bile-pancreatic duct (BPD). He found that the rat BPD acted in a similar way to the main pancreatic duct of the cat with respect to its ionic permeability of HCO_3^- and Cl^-. The structure of the BPD wall in the rat is very similar to that of the feline pancreatic duct and thus one would expect ionic diffusion to behave in a similar manner. However, cannulation of the rat BPD is difficult and Olazabal (1983) failed to characterize his basic preparation. Nevertheless, this preparation offered possibilites which we were able to exploit fully later in this study. Rohr (1983) further studied the permeability of the rat BPD to exocrine pancreatic enzymes using an experimental preparation similar to that of Olazabal. Under basal conditions the duct appeared relatively impermeable to these enzymes whereas after the induction of acute pancreatitis this permeability was increased three- to four- fold.

Several methods of assessing pancreatic duct integrity, or barrier function, are available. The simplest methods are those of measuring anionic flux of chloride and bicarbonate across a given length of duct in a specified time. Determination of transductal potential difference (a measure of HCO_3^- permeability) is an adjunct to ionic flux. Ultrastructural assessment of the epithelial cells is as important as measurement of ionic flux. The most complete evaluation of pancreatic duct integrity is that using a combination of ionic flux, potential difference and mucosal ultrastructure. When these indices are used in conjunction a true quantification of the degree of duct damage can be made.

O'Leary and associates (1982) have recently studied pancreatic duct permeability using intraductal injection of diatrizoate meglumine followed by serial computerized tomography scanning. They concluded that pancreatic contrast disappearance rates provided a measure of pancreatic duct epithelium permeability and the rates obtained were consistent with a process of passive diffusion. However, there are several criticisms of this study as no pressure measurements were made, the contrast itself may damage the duct (Bub 1983) and the method is insensitive to small changes in permeability.

It must be emphasized that by measuring ionic flux of Cl^- (mol. wt. 30) and HCO_3^- (mol. wt. 61), these ions are acting as simple markers of duct permeability. There is now evidence that macromolecules can cross the damaged duct wall and these substances are themselves potentially damaging to the acinar cells once extravasation has occurred. Konok and Thompson (1969), in perhaps the pioneering study on the pancreatic duct mucosal barrier , used the molecule curare (mol. wt. 785) as a marker of duct integrity. Normal duct mucosa is impermeable to curare whereas damaged mucosa is extremely permeable. Later, Mosley 1981 demonstrated that bilirubin (mol. wt. 584) was able to diffuse across the duct once its integrity had been compromised. It was Reber and colleagues (1981b, 1982) however, who fully evaluated the permeability of the pancreatic duct to macromolecules. In their first study (1981b) they used fluorescently labelled dextrans of known molecular weight (3,000, 20,000, 40,000) to study the transmission of large molecules across the pancreatic duct into the portal blood, before and after exposure of the duct to a bile salt. With the normal duct no macromolecules were able to cross. Once the duct had been damaged by exposure

to bile salt, the duct was made permeable to molecules up to 40,000 mol. wt. Their further reports (Reber 1982, Wedgewood 1984) used a similar experimental method and determined that the feline main pancreatic duct became permeable to molecules as large as 20,000 daltons after bile salt exposure. Further work by Austin (1984) has demonstrated that high pressure increases macromolecule movement across the damaged duct wall. These observations on macromolecular permeability are important as the pancreatic enzymes that have been implicated in the pathogenesis of acute pancreatitis are of similar size e.g. phospholipase A_2 14,800, trypsin, chymotrypsin and elastase 25,000. Such diffusion of toxic substances out of the duct into the pancreatic parencyma after damage to the PMB might well be important in the pathogenesis of acute gallstone pancreatitis. Indeed this diffusion of enzymes from the damaged duct has been recently demonstrated by Rohr (1983).

ERCP examination of the human pancreatic duct is commonly performed and the contrast agents now used are of low toxicity althoughsome complications still develop (Bilbao 1976). Bub and co-workers (1983) have recently described the morphology of the pancreatic duct epithelium after pancreatography with various contrast media. The duct epithelium was markedly damaged after injection of several contrast media and this damage was exacerbated by high pressure. It is of interest that the cellular damage was similar in nature to that observed after disruption of pancreatic duct integrity. Damage to the PMB by contrast agents may lead to increased duct permeability and thus be responsible for post ERCP hyperamylaseamia and acute pancreatitis.

Altered pancreatic duct permeability has been implicated in the

pathogenesis of both gallstone pancreatitis and adenocarcinoma of the pancreas. O'Leary and colleagues (1984) have investigated pancreatic duct epithelial characteristics after bile salt induced damage. They induced severe epithelial dysplasia and suggested that a membrane mediated process was involved with possible changes in membrane permeability at the luminal surface. Their findings suggest that studies of pancreatic duct permeability might relate to the pathogenesis of pancreatic cancer. Furthermore, as carcinoma of the pancreas is more commonly found in the head and ampullary areas of the gland, refluxed bile or duodenal contents might cause damage to pancreatic duct integrity and thus permit carcinogens to exert their toxic effects.

Damage to pancreatic duct integrity, associated with compromise to the pancreatic duct mucosal barrier, has been experimentally induced by several compounds. These substances are similar to those known to break the gastric mucosal barrier and are presumably surface membrane attackers. Amongst the substances shown to damage the PMB are

- Infected bile (Konok and Thomspon 1969)
- Bile salts (Reber 1979, 1980)
- Aspirin (Mosley 1979, Fox 1979)
- Ethanol (Reber 1979)
- Trauma by indirect cannulation (Case 1970).

The effects of these agents on barrier function will be discussed at length in the following chapters.

Several conclusions can be drawn from these observations.

1. The pancreatic ducts are analogous to the stomach in possessing a physiological barrier to free diffusion.

2. Damage to this barrier is associated with increased anionic flux, reduced potential difference and ultrastructural changes in the duct mucosa.
3. Damage to duct integrity makes the duct permeable to macromolecules which may have relevance in the pathogenesis of acute pancreatitis and pancreatic neoplasia. Chloride and bicarbonate ions are only markers of this damage.
4. The feline pancreatic duct has been used for most studies. As experimental economies are now of importance in most laboratories there is a need to evaluate the rat bile-pancreatic duct.

Fig. 25A Electron microscopy of normal duct (x 7500).

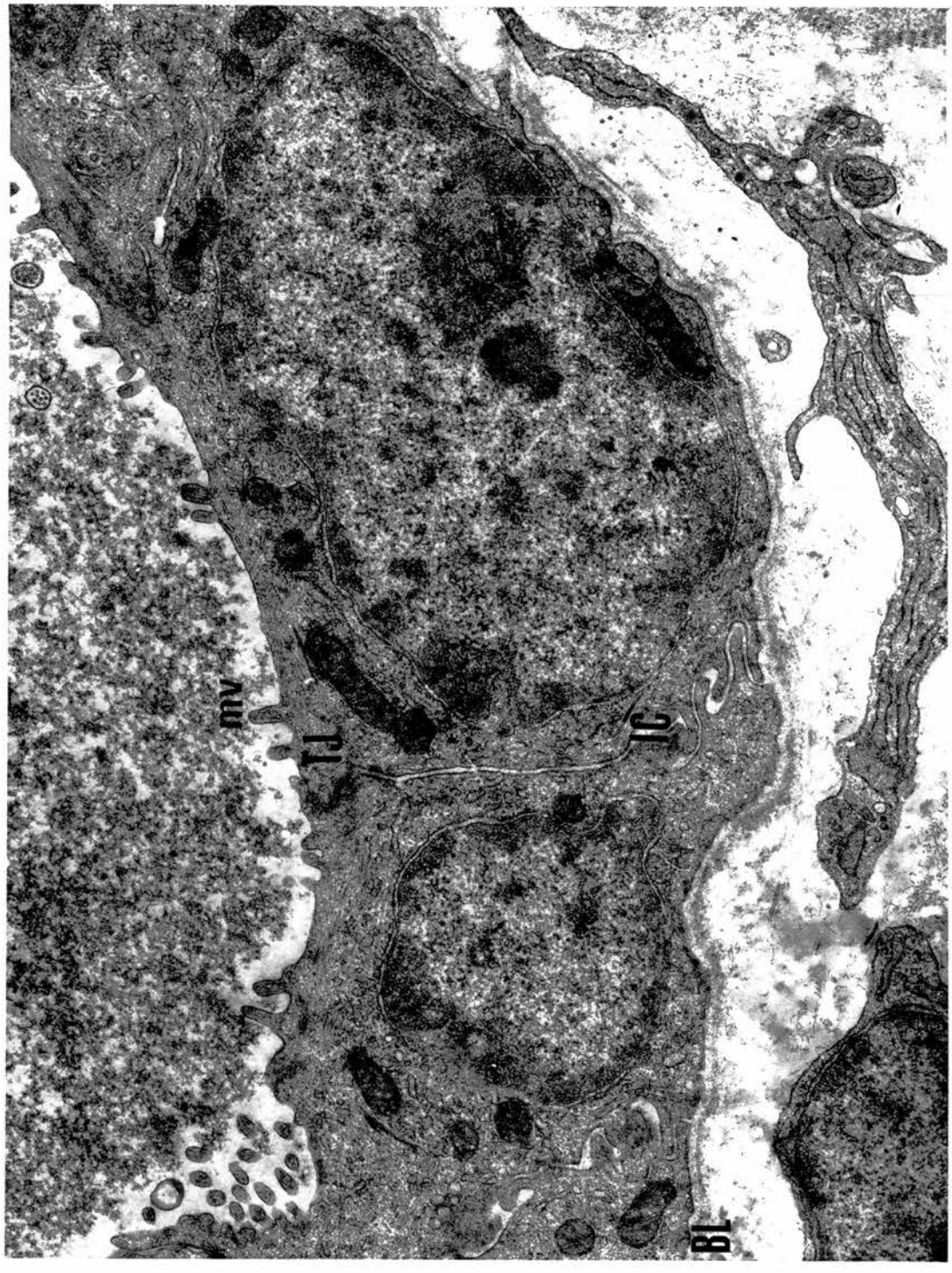


Fig. 25B Electron microscopy of normal duct (x 7500).

B.L. - Basement lamina
mv - microvilli.
T.J.-tight junction.
IC - intercellular space.

Fig. 26 Electron microscopy of tight junction (75,000).
T.J.- tight junction.
mv - microvilli.

Materials and Methods

Experimental preparation

Fasting male Sprague-Dawley rats (250-300g) were anaesthetized with intraperitoneal Inactin (100 mg/kg) (sodium thobutabarbitone; BYK, Germany) and kept at a constant temperature of 37°C. The animals were placed on a sheet of aluminium foil to give electrical shielding. The external jugular vein and internal carotid artery were cannulated to allow intravenous infusion, measurement of potential difference and monitoring of blood indices. The blood levels of Cl^- and HCO_3^- remained in the ranges 100-110 mmol/l and 23-30 mmol/l respectively throughout the experiment. Tracheostomy was performed in all animals to maintain a patent airway.

The abdomen was opened along the midline and the bile-pancreatic duct (BPD) ligated close to the liver (fig. 27A-D). A thin polyethylene tube (Portex, id 0.4 mm, od 0.8 mm) was inserted into the duct distal to the tie and ligated in place. Transduodenal cannulation of the distal BPD with a similar cannula was performed and the cannula lightly tied in place. At all times care was taken to avoid damage to the duct or the pancreatic acini. The distance between the tips of the two cannulae was carefully measured and ranged from 1.7 to 2.3 cm (mean 2.1 cm).

The distal cannula was connected to a constant infusion pump (slow infusion apparatus, Scientific and Research Instruments Ltd, Croydon, U.K.). which allowed perfusion at varying rates. A pressure transducer (Bell and Howell, type 4/422, Basingstoke, England) was incorporated via a 3-way tap into the infusion system to allow constant monitoring of pressure.

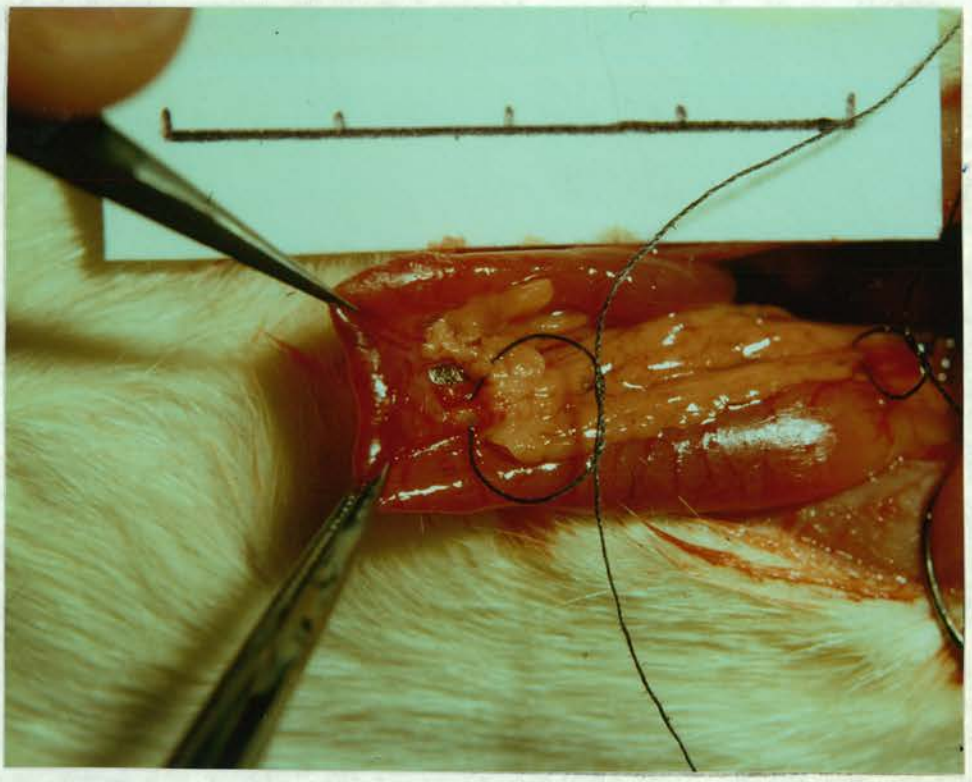


Fig. 27A Ligatures around distal and proximal bile-pancreatic duct (scale in centimetres).

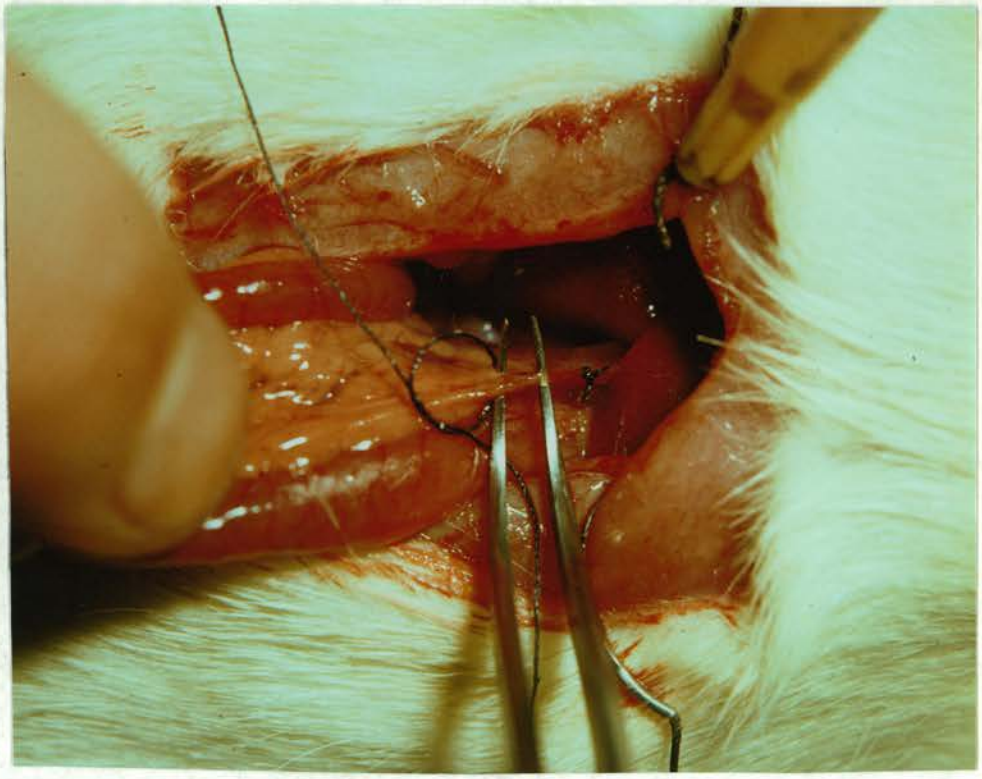


Fig. 27B Cannulation of proximal bile-pancreatic duct.

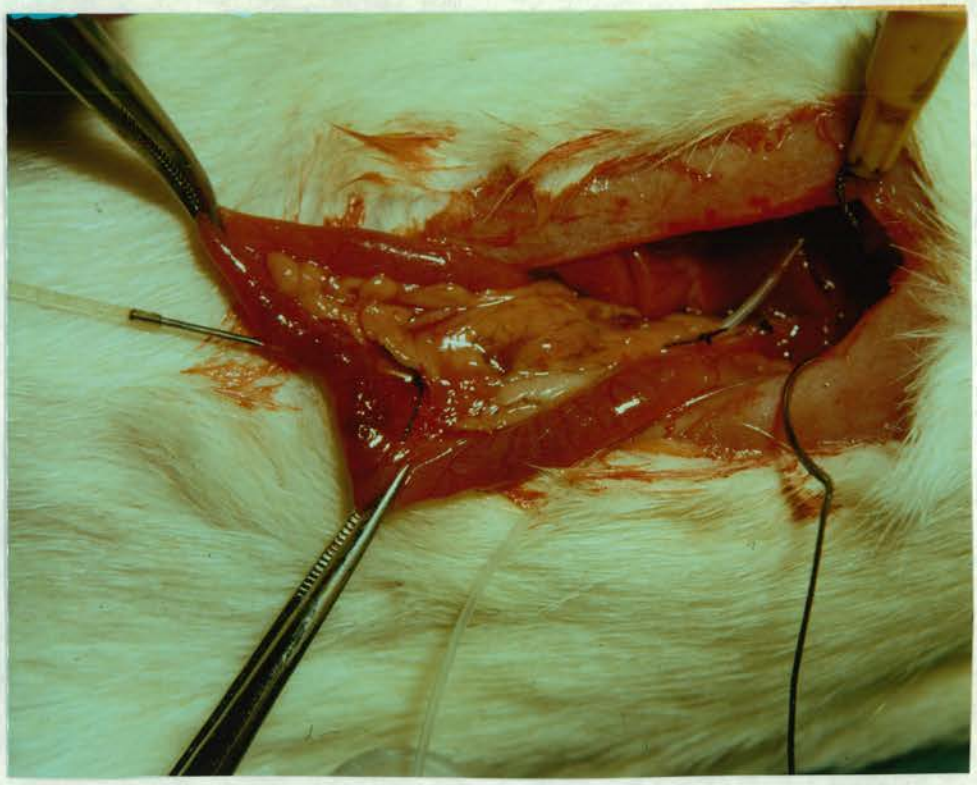


Fig. 27C Cannulation of distal and proximal bile-pancreatic duct.

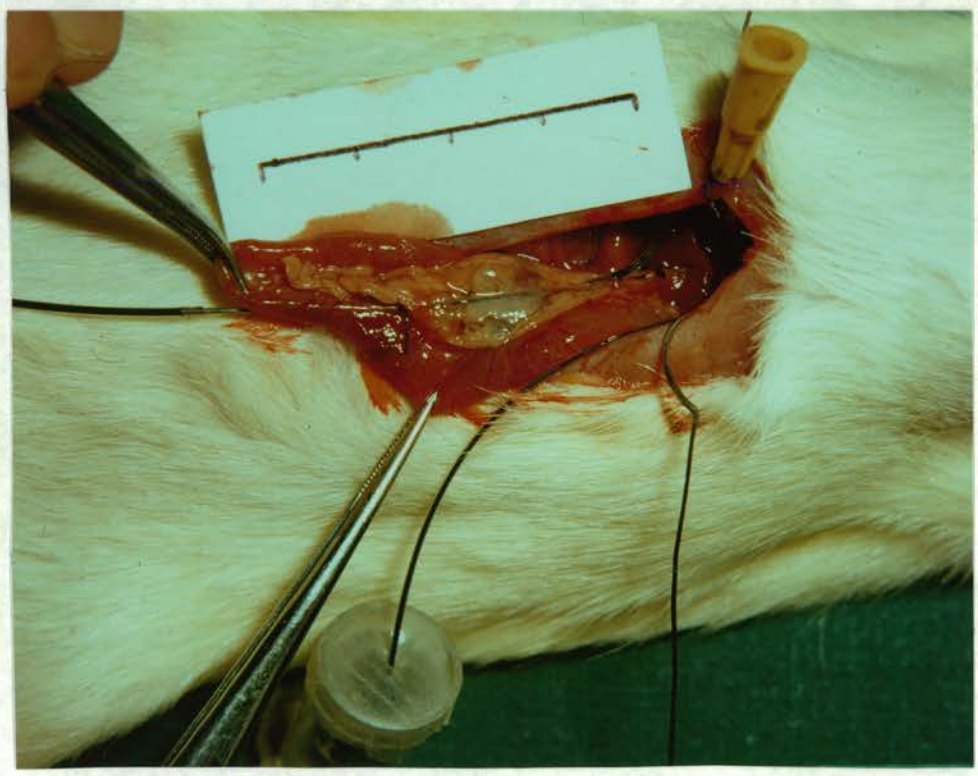


Fig. 27D Perfusion of duct. Indian ink used for clarity.

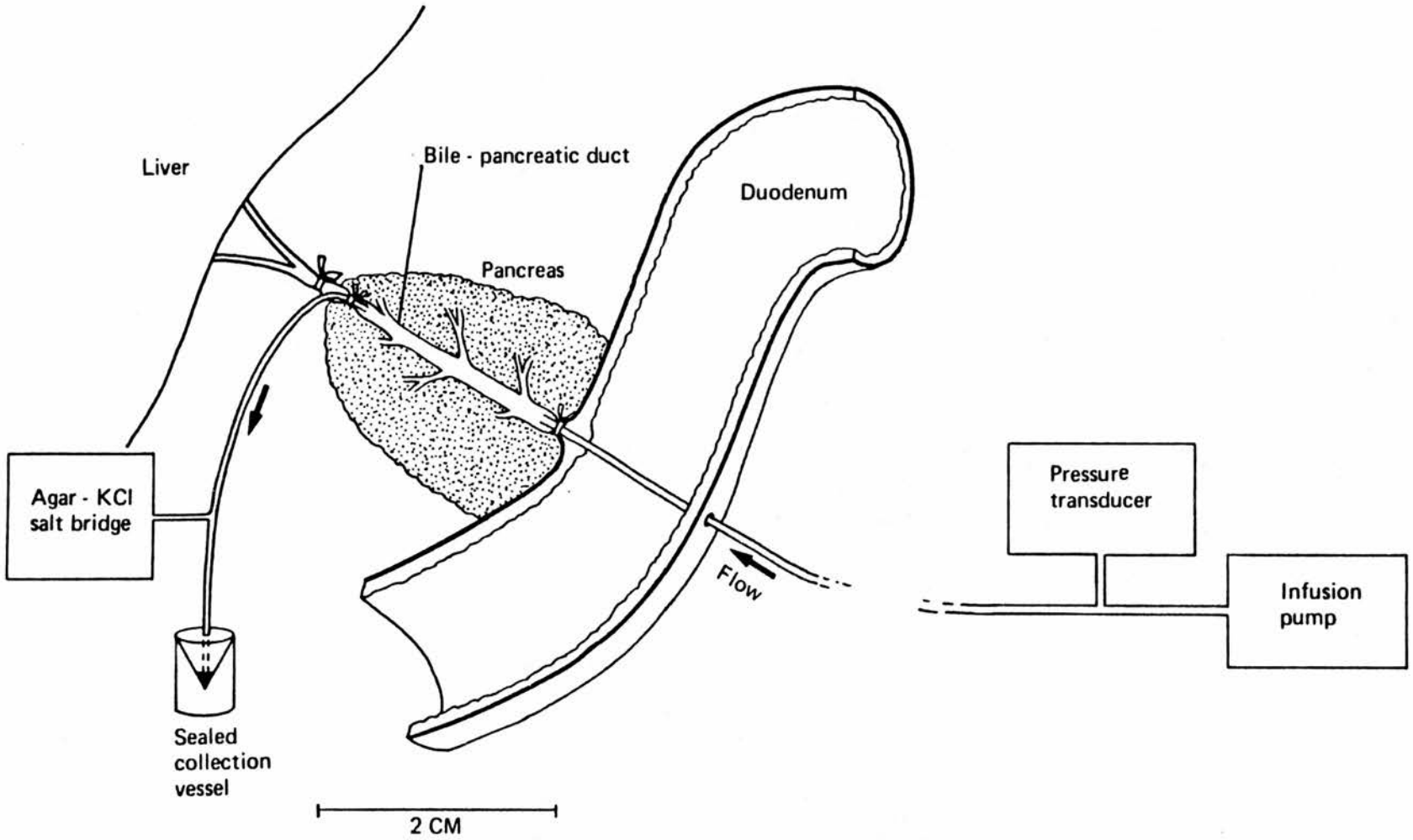


Fig. 28 Experimental preparation for perfusion of duct.

Effluent was collected in small containers under paraffin to prevent evaporation. The effluent cannula incorporated one salt bridge (see later). The completed experimental preparation is shown in fig. 28.

Solutions

The standard perfusate solution (SPS) had the following composition:

HCO_3^- - 120 mmol/l.

Cl^- - 30 mmol/l.

Na^+ _ 150 mmol/l.

pH - 8.4.

i.e. anionic concentration close to that of pancreatic juice.

Osmolality - 300 m osmol/kg; the same as rat plasma. Adjusted by the addition of mannitol.

SPS was made up freshly before each experiment and anionic concentrations measured before use.

Anionic measurement

Chloride concentration was determined using a CM T10 chloride titrator (Radiometer, Copenhagen, Denmark). 10 µl volumes were used for analysis. Bicarbonate concentration was determined using a Natelson microgasometer. All estimation were performed within two hours of the specimen being obtained.

Potential difference

The effluent cannula of the perfusion system (fig. 28) incorporated one salt bridge of saturated KCl in 4% agar which made electrical

contact with the fluid in the collection catheter. The other salt bridge was inserted into the external jugular vein. The opposite end of each bridge was immersed in a beaker of saturated KCl containing a calomel electrode. These were connected to a Farnell type TM 39 microvoltmeter and a pen recorder for continuous recording of transductal potential difference (pD).

Spontaneous pancreatic secretion

The basal pancreatic secretion volume was measured in eight rats. The values for the first and second hour collection periods were

first hour - 4.6 ± 0.59 µl/hr (mean ± SD)

second hour - 4.8 ± 0.82 µl/hr

Electron microscopy

At the end of the experiment and prior to sacrifice the BPD was perfused with 4% glutaraldehyde in Sorensen's buffer pH 7.4 for 10 minutes at a rate of 0.2 ml/hr. The duct was then excised and stored in fixative for 48 hours. A segment of the duct was postfixed in 1% osmium tetroxide, and then dehydrated in ethanol prior to embedding in Emix resin (Emscope Ltd., Ashford, Kent, UK). A 2 µm section was taken from the centre of the block, stained with 1% toluidine blue in 1% sodium tetraborate, and examined under the light microscope to ensure correct orientation of the duct and optimum sampling. Ultrathin (50 nm) sections were then cut and stained in lead citrate for 5 minutes. The stained sections were examined in a Phillips 300 electron microscope and recorded on photographic film.

Study Design

On the basis of the later perfusion rate observations a rate of perfusion of 100 µl/hr was used for anionic flux studies. At perfusion rates above this, exchange of ions was low and at rates below this there was insufficient volume of effluent to perform the biochemical analyses.

To perform the perfusion experiments the following order of events was used.

Period	Perfusate	Rate (µl/hr)	Time (mins)	Effluent
	SPS	1000	10	discard
	SPS	100	20	discard
I	SPS	100	60	collect
II	test solution (e.g. bile, bile salts etc)	200	20	discard
	SPS	1000	10	discard
	SPS	100	20	discard
III	SPS	100	60	collect
	glutaraldehyde	200	10	duct removed

The experiment was continued after the first hour of infusion only if the volume of the fluid collected by that time (measured by weighing, 1 g = 1 ml) was 100 ± 5% of the infused volume. Less than 2% of the experiments were discarded because of poor recovery (defined as $<95\%$ of the infused volume).

Animal stability

The total length of the experiment including the initial surgery

was approximately 240 minutes. During this time the animals were stable with Inactin anaesthesia and required no further doses. Inactin is well known as a particularly useful anaesthetic agent (Sewell, 1975), with the only dangers being at induction when sudden death can occur. The blood indices of H^+, Cl^-, HCO_3^-, pO_2 and pCO_2 all remained within the normal physiological range. Every 30 minutes the trachea was aspirated to prevent mucus accumulation. Once the preparation was established no animal died during the experimental period.

Ion flux

Differences between the Cl^- and HCO_3^- concentrations of the effluent and perfusate were determined for periods I and III. Flux is represented by the letter J and was calculated in the following manner

$$J.\ Cl^- = \frac{[Cl^-]\ \text{effluent} - 30}{\text{length duct}} \times \frac{1}{10}\ \mu\text{mol/cm/hr};\ 30 = [Cl^-]\ \text{perfusate}$$

$J.\ Cl^-$ = +, i.e. gain of ions into duct lumen

$$J.\ HCO_3^- = \frac{[HCO_3^-]\ \text{effluent} - 120}{\text{length duct}} \times \frac{1}{10}\ \mu\text{mol/cm/hr};\ 120 = [HCO_3^-]\ \text{perfusate}$$

$J.\ HCO_3^-$ = -, i.e. loss of ions from duct lumen

Small values for ion flux correspond to low duct permeability and high values to high permeability.

The mean changes in ionic flux, i.e. the difference in flux between periods III and I, were evaluated as (Δ = change).

$$\Delta J.\ Cl^- = J.Cl^-_{III} - J.Cl^-_{I}\ (\mu\text{mol/cm/hr})$$

$$\Delta J.\ HCO_3^- = J.HCO_{3III}^- - J.HCO_3^-\ I\ (\mu\text{mol/cm/hr})$$

Differences in ionic flux between period III and I were interpreted as being due to differences in the permeability of the duct caused by damage from the agent perfused during period II. A small mean change

in ionic flux represented duct stability between periods III and I. The larger the mean change the more marked was the effect of the test substance on duct permeability.

An example of the calculations employed is given below

e.g. length duct 2.0 cm.

perfusate $[Cl^-]$ 30, $[HCO_3^-]$ 120 mmol/l.

period I effluent $[Cl^-]$ = 45, $[HCO_3^-]$ = 90.

period II Bile infused.

period III effluent $[Cl^-]$ = 60, $[HCO_3^-]$ = 70.

$\therefore$ for period I: $JCl^- = \frac{45 - 30}{2.0} \times \frac{1}{10}$

$= +0.75$ µmol/cm/hr.

$JHCO_3^- = \frac{90 - 120}{2.0} \times \frac{1}{10}$

$= -1.50$ µmol/cm/hr.

likewise for period III $J.Cl^- = +1.50$ µmol/cm/hr.
$J.HCO_3^- = -2.50$ µmol/cm/hr.

$\therefore$ mean change in ion flux (period III-period I)

$\Delta J.Cl^- = 1.5 - 0.75 = +0.75$ µmol/cm/hr
$\Delta J.HCO_3^- = -2.5 - -1.50 = -1.0$ µmol/cm/hr

} These values indicate marked damage to PMB.

Potential difference (pD)

The transductal pD was determined in periods III and I and the change in pD between these two periods was calculated as

pD change (Δ.pD) = $pD_{III} - pD_I$

The larger the change in pD the greater the damage produced by the test substance.

PERMEABILITY OF THE RAT BILE-PANCREATIC DUCT

Before proceeding to evaluate the effects of varying substances on the bile pancreatic duct (BPD) it was essential to study the physiological characteristics of this preparation. The purpose of these experiments was (i) to establish what conditions should be used for further experiments and (ii) would this rat preparation behave in a similar way to that previously described using the feline main pancreatic duct.

Perfusion rates

SPS was perfused through the duct at variable rates ranging from 50 to 250 µl/hour, and five animals were perfused at each rate. The experimental result was only accepted if the collected effluent volume was within 5% of the perfusate volume. The anionic (Cl^-, HCO_3^-) concentration was determined in the effluent and recorded against perfusion rate (fig. 29). When the BPD was perfused with SPS at rates greater than 200 µl/hr, the concentrations of HCO_3^- and Cl^- in the recovered effluent were unchanged, i.e. the SPS was flowing through the duct too quickly to allow ionic permeation. As the flow rate decreased the concentration of Cl^- was higher and HCO_3^- lower in the effluent than in the perfusate, indicating flux of anions across the duct wall. At the low rate of perfusion of 50 µl/hr almost complete ionic equilibration occurred. On the basis of these results a perfusion rate of 100 µl/hour was used for all future flux studies for the following reasons

(i) at higher rates there was insufficient flux

(ii) at lower rates - the volume of effluent was too low to perform estimations.

- the volume of basal pancreatic secretion might become significant.

- ion flux was so high in the control conditions that changes would be difficult to interpret.

Perfusion pressure

The pressures in the duct system at the various rates of perfusion are illustrated in table 21. With a flow rate of 100 µl/hr the pressure never exceeded 9 cm H_2O. As the flow rate increased so did the pressure until values of 28.3 cm H_2O were obtained with a flow rate of 250 µl/hr. The perfusion rate of 100 µl used for these ion flux studies is therefore associated with low ($<$ 10 cm H_2O) pressures.

Anionic movement

The theory that HCO_3^- and Cl^- diffuse across the duct epithelium down their respective concentration gradients was tested by substituting the observations (from the perfusion rate vs. ion flux experiment above) in an integrated form of the Fick equation described by Defares and Sneddon (1960) and later by Case and Scratcherd (1970).

i.e.

$$\text{Ln}\ (u - a) = \text{Ln}\ (u_o - a) - 2\pi rlh \cdot \frac{1}{V_o}$$

where Ln = natural logarithm.

u_o = ionic concentration of perfusate.

u = ionic concentration of effluent.

a = ionic concentration of extracellular fluid

Cl^- = 110, HCO_3^- = 26 mmol/l.

r = radius duct.

l = length duct.

h = permeability coefficient.

v_o = flow rate.

In any one experiment only u and Vo are variable. Therefore a graph of Ln (u - a) against $\frac{1}{Vo}$ will be linear if Fick's law holds. The slope of the line obtained is $2\pi rlh$ and therefore any difference in gradient is due to a difference in permeability of the duct to the two ions (Cl^-, HCO_3^-).

The observed values of effluent ionic concentration (u) and flow rate (Vo) were substituted into the equation above. The graphs of Ln (u - a) against $1/V_o$ for Cl^- and HCO_3^- are illustrated in fig. 30 and the points for Cl^- (r = 0.96) and HCO_3^- (r = 0.998) fall on straight lines. These results indicate that both Cl^- and HCO_3^- move across the duct wall by passive diffusion down concentration gradients and conform to the DeFares and Sneddon (1960) equation. The gradients of the two lines were different; Cl^- = -0.04, HCO_3^- = -0.06, indicating that the BPD of the rat had a higher permeability to HCO_3^- than Cl^- ions.

Volume recovery

To evaluate completeness of volume recovery in this preparation 11 rats were infused with SPS containing 5 uCi/ml of ^{14}C polyethylene glycol (PEG, mol. wt. 4000, Radiochemical Centre, Amersham, Kent). The duct was perfused at varying rates with SPS and PEG for 1 hour and the effluent collected. The duct was then perfused with unlabelled SPS (wash solution) for 20 minutes and the effluent collected. The specific activities of labelled perfusate (cpm_p), effluent (cpm_e) and effluent following wash perfusion (cpm_w) were measured using a Packard liquid Scintillation Counter (tri-carb 460 C, Packard Instrument Company Inc., Downes Grove, Ill., USA). The effluent volume was measured by weight and compared with the volume calculated from the specific activity in the perfusing solution and effluent. Recovery of ^{14}C PEG (%) was calculated according

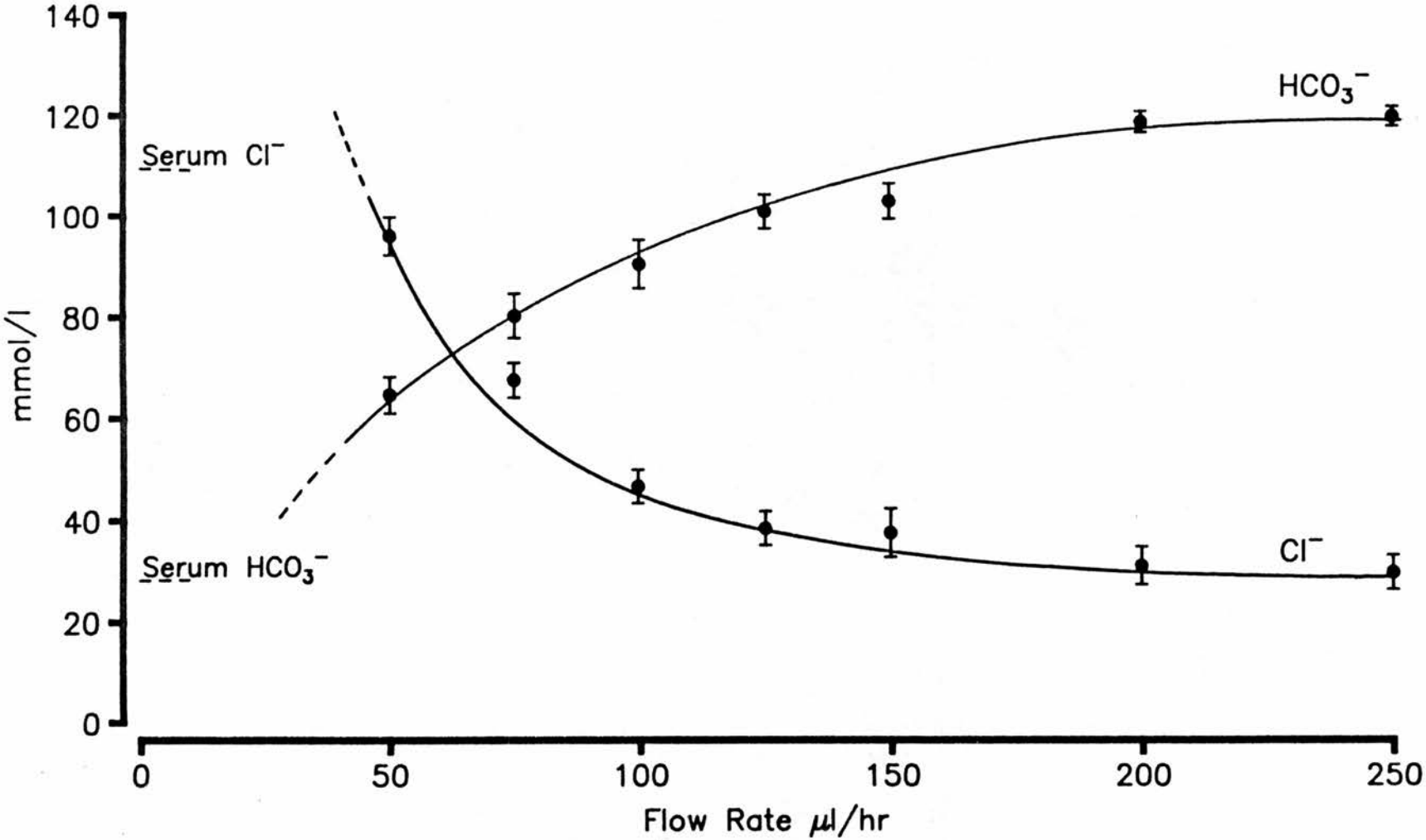


Fig. 29 Effluent anionic concentration vs. flow rate for Cl^- and HCO_3^- anions.

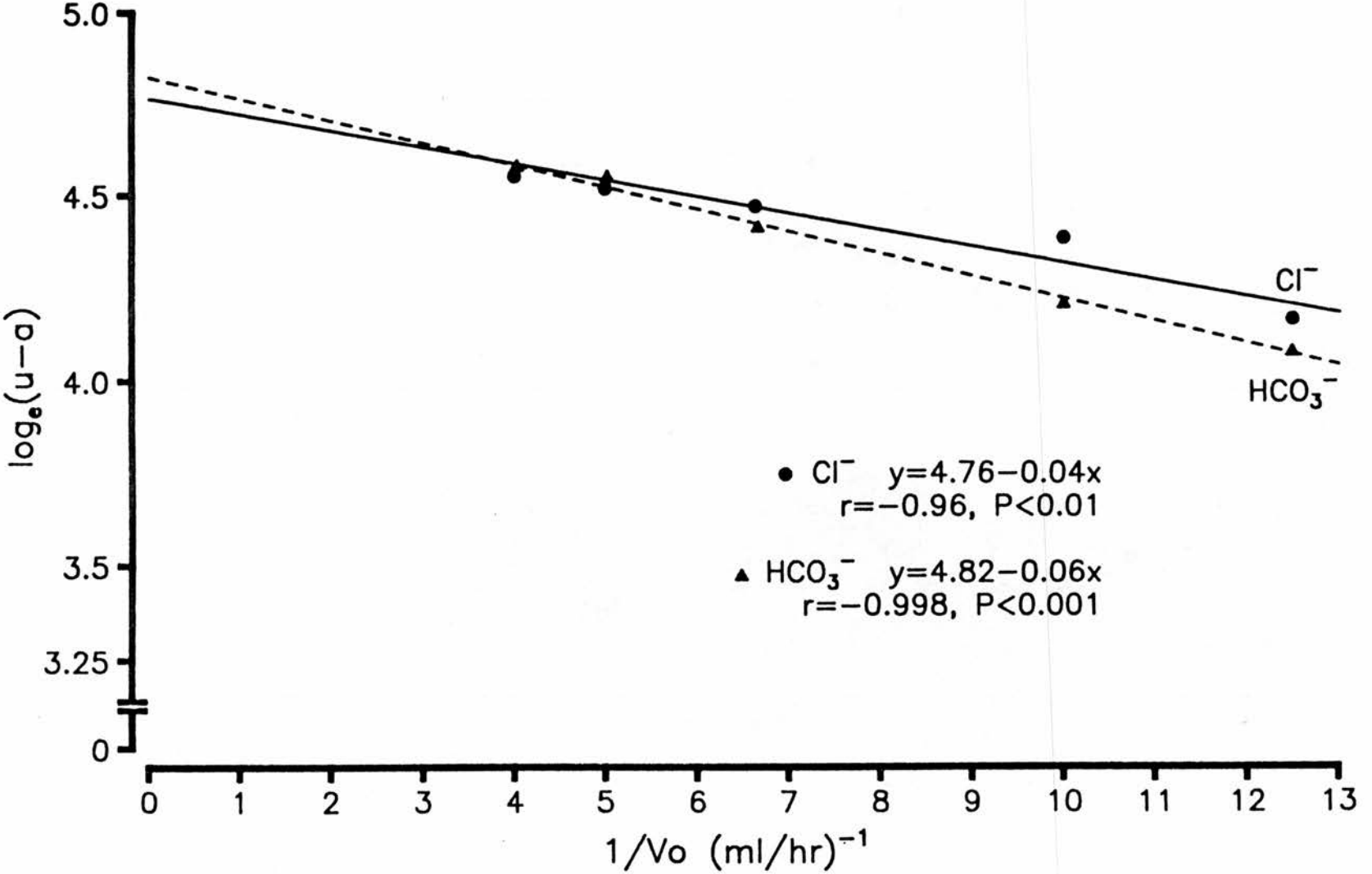


Fig. 30 $\log_e$ (u-a) vs. 1/Vo; values substituted into equation of Defarres and Sneddon.

TABLE 21 Intraductal pressure at varying rates of perfusion (mean ± SD).

	PERFUSION RATE (µl/hr)			
	100	150	200	250
pressure (cm H_2O)	7.3±1.2	12.7±2.5	18.7±1.7	28.3±2.9

to the method of Simpson (1983) as

$$\% \text{ recovery} = \frac{Ve \times cpm_e}{Vp \times cpm_p} \times 100$$

Ve = measured volume effluent.

Vp = measured volume perfusate.

The percentage recovery of ^{14}C PEG following wash perfusion was calculated.

$$\% \text{ recovery} = \frac{Vw \times cpm_w}{Vp \times cpm_p} \times 100$$

Vw = volume of wash solution recovered after 20 minutes.

The percentage recovery of ^{14}C PEG at various flow rates is shown in table 22. Most of the infused ^{14}C PEG (>94%) was collected in the effluent indicating that sequestration (5% in wash) was of minor importance. The relationship between calculated ^{14}C PEG volume and observed volume recovery at the end of 1 hour of recovery is illustrated in fig. 31. There was close correlation between the values ($r = 0.989$, $P < 0.0001$) implying that virtually all the perfusate volume was collected in the effluent and there was little sequestration of fluid.

Potential difference (pD).

The relationship between the transductal pD and the $[HCO_3^-]$ difference between perfusate and serum was studied in twenty animals. In these experiments the perfusate concentration of HCO_3^- was varied from 10 to 140 mmol/l and that of Cl^- from 140 to 10 mmol/l. The total anionic concentration in the perfusate was always 150 mmol/l. The transductal pD was measured continuously during the perfusions, and the serum $[HCO_3^-]$ was determined at intervals.

There was a linear relationship between the pD across the duct and the

TABLE 22 Percentage recovery of ^{14}C PEG following BPD perfusion at varying flow rates (mean values given).

PERFUSION RATE (µl/hr)

	100	150	200	250
No. of rats	4	3	2	2
% recovery ^{14}C PEG in effluent	97.2	96.3	94.2	95.1
% recovery ^{14}C PEG in wash solution	4.6	3.8	5.1	6.4

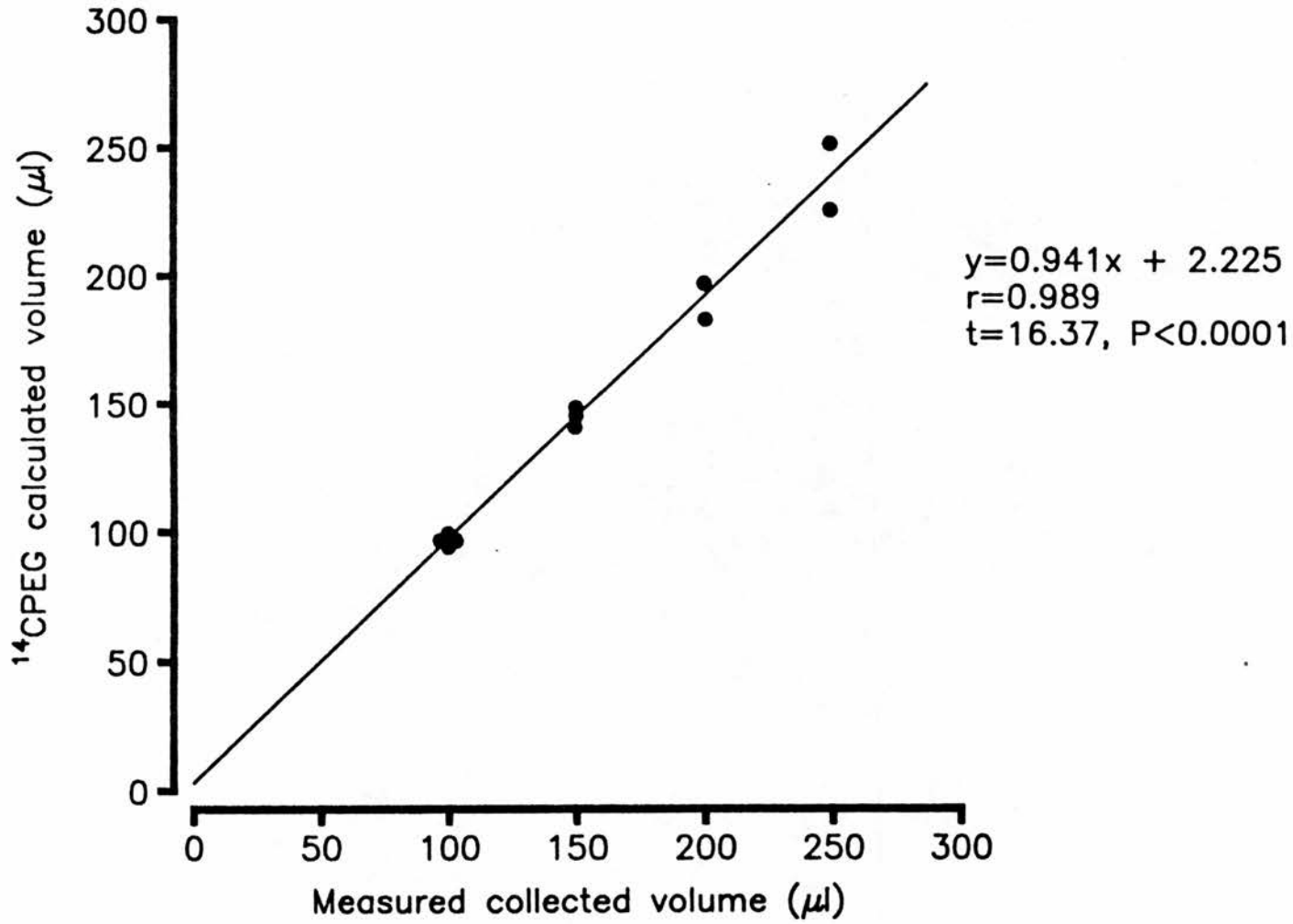


Fig. 31 Calculated (^{14}C PEG)volume vs. measured volume.

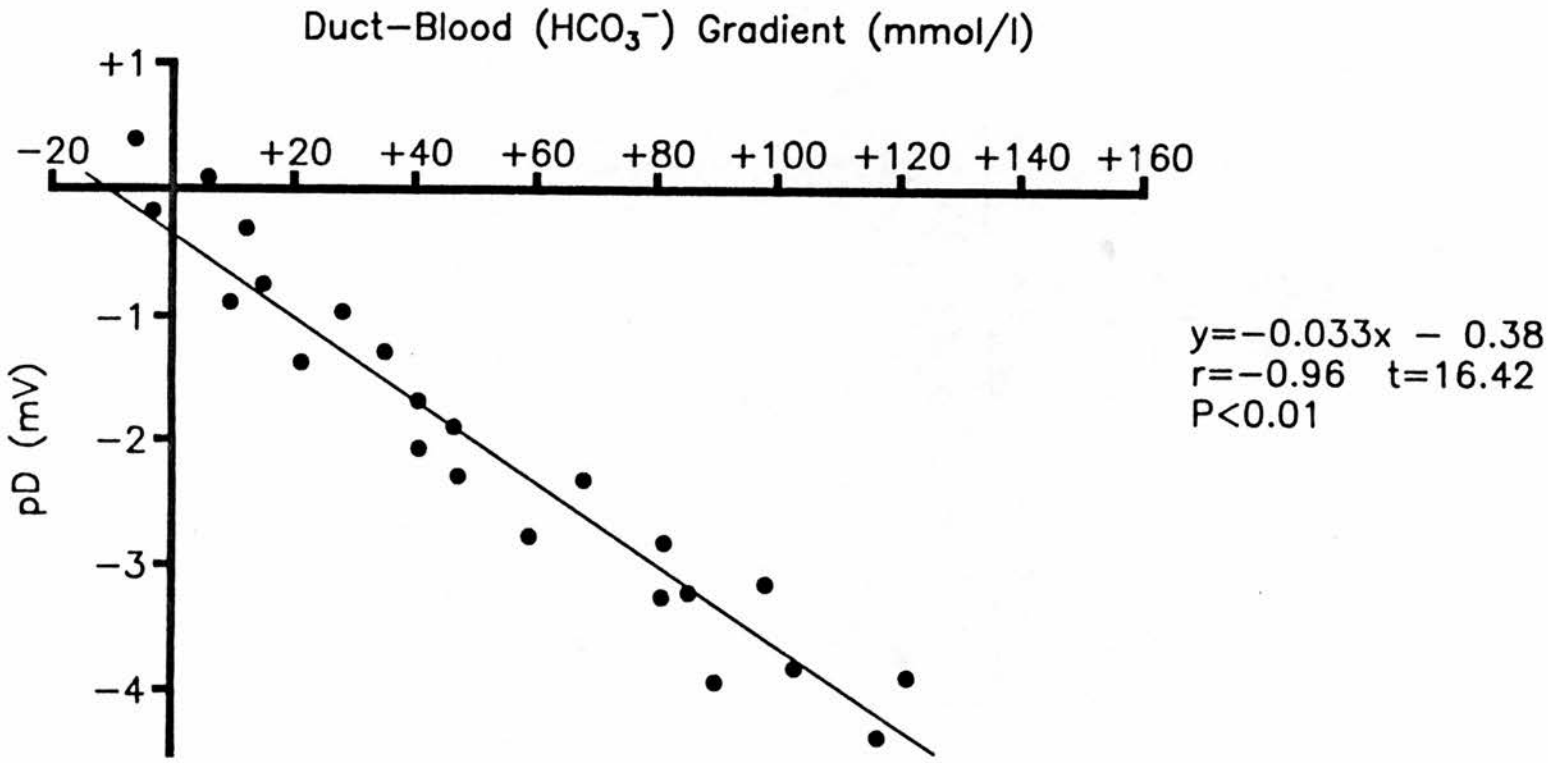


Fig. 32 Transductal pD vs. $[HCO_3^-]$ gradient.

difference in $[HCO_3^-]$ between the perfusate and serum (fig. 32). When the perfusate $[HCO_3^-]$ was 100 mmol/l the pD was -3.6 ± 0.21 mv. When the perfusate $[HCO_3^-]$ was the same as the serum $[HCO_3^-]$, the pD was -0.3 ± 0.2 mv, close to zero. This linear relationship was statistically significant ($r = -0.96$, $P < 0.01$).

Control anionic flux and pD

Studies of anionic flux and pD were performed at a perfusion rate of 100 µl/hr. The values for period I and period III are given below (period II = SPS only, or control).

	period I (N = 165)	period III (N = 46)
$J.Cl^-$ (µmol/cm/hr)	+0.84 ± 0.10	+0.85 ± 0.11
$J.HCO_3^-$ (µmol/cm/hr)	-1.59 ± 0.12	-1.58 ± 0.14
pD (mV)	-2.3 ± 0.11	-2.28 ± 0.07
		(mean ± SD)

These results demonstrated the stability of the duct over the experimental period.

Control electron microscopy

The epithelial lining of the normal bile-pancreatic duct is illustrated in figs 25A and B. There is a single layer of columnar cells resting on a continuous basal lamina. Occasional side branches could be seen joining the main duct. The supporting connective tissue contained striated bundles of collagen, occasional fibroblasts and small capillaries. The columnar cells were joined at the apex by typical junctional complexes (fig. 26). Moderate numbers of surface microvilli extended into the

duct lumen. The cytoplasma contained various numbers of mucus secretory granules, mitochondria,endoplasmic reticulum and a Golgi complex. The lateral membranes of adjacent columnar cells were closely opposed with narrow intercellular spaces occupied by short interdigitating processes. Desmosomes were occasionally seen in the upper portions, just beneath the apical junctional complex and there was no connection between the basal lamina and basal portion of the columnar cell. Several endocrine like cells, containing typical secretory granules, were seen at intervals in the epithelium.

Fixation of tissues is of vital importance in interpreting electron microscopy and this was excellent in the sections examined. Control animals, given SPS in period II, demonstrated normal duct ultrastructure at the termination of the experiment. The only small abnormality was a slight increase in the width of the intercellular spaces. In particular, there was no evidence of cell damage, as evidenced by normal intercellular organelles and normal junctional complexes.

Results summary

1. The rat bile pancreatic duct is analogous to the main pancreatic duct of the cat in possessing a "barrier" to free diffusion of Cl^- and HCO_3^- ions.
2. The rat bile pancreatic duct has similar properties to the feline pancreatic duct. Anionic movement is by slow passive diffusion down concentration gradients.
3. The preparation is stable during the period of study and there is over 94% volume recovery.
4. The permeability of the BPD wall can be assessed by measuring flux of Cl^- and HCO_3^- and transductal pD.

5. The results obtained are highly reproducible between animals.
6. Electron microscopy is a useful adjunct to anionic flux and pD in assessing duct integrity.
7. This preparation offers opportunities for the study of pancreatic duct integrity in response to various injurious agents.

Discussion

Investigation of duct integrity using the rat bile pancreatic duct is a new concept in evaluating pancreatic disease. This study has demonstrated the efficacy of the in situ model of bile-pancreatic duct (BPD) perfusion in the investigation of pancreatic duct physiology. The stability of the animal preparation was confirmed by the constancy of ionic flux measurements and the absence of change on ultrastructural examination. Over the four hour period of the experiment the anaesthetic used (Inactin) permitted a constant physiological environment - the blood indices were constant, no further anaesthesia was needed and all vital signs remained stable. Indeed the use of the anaesthesia Inactin has now become widespread in long-term physiological experiments; up to 24 hours with a single dose (Sewell 1975). It is important to emphasize that this study used direct cannulation of the BPD, as indirect cannulation has been shown by Case (1970) to damage the pancreatic duct mucosal barrier. Moreover care was taken at all times to avoid trauma to the pancreatic duct system.

Studies on the cat pancreas have demonstrated that the HCO_3^- and Cl^- concentrations in the secreted fluid are dependent on the flow rate whereas the concentrations of sodium and potassium remain constant at

all secretory rates (Case 1969, 1970). At high flow rates the pancreatic juice contains a high HCO_3^- and low Cl^- concentration, the reverse being the case with low flow rates. Thus the pancreatic ducts act as secondary modifiers of acinar secretion.

The preparation described in this study has demonstrated an exchange of anions that was dependent on perfusion rate. The only similar study in the literature is that of Olazabal (1983a) who demonstrated anionic exchange to be more than that obtained in the present investigation. The discrepancy in results may be explained by; different methodology as Olazabal used 'sagatal' anaesthesia which requires constant 'topping up'; the length of ductal perfusion was only 1.4 cm as opposed to 2.0 cm in this study; and the direction of perfusion was different to that employed here. This report has shown that at flow rates of greater than 250 µl/hr, negligible exchange of anions occurred, presumably as a result of difficulty in detecting the small amounts of HCO_3^- and Cl^- moving across the mucosa relative to the greater mass of ions passing through the system (Simpson 1983). As the flow rate decreased so did the anionic flux increase. The graph of flow rate against effluent anionic concentration is similar to that obtained in studies of the feline pancreatic duct (Reber 1979, 1980, Case 1969, 1970) although because of the size of the rat the volumes are correspondingly smaller. Indeed the movement of anions in the rat BPD appears to be of a similar magnitude to that in the pancreatic duct of the cat. This study used the perfusion rate of 100 µl/hr, as did Olazabal (1983), since the ionic fluxes obtained were large enough to detect significant differences. A comparison of the perfusion details for feline and murine experiments is given below.

	Body size	length duct	perfusion rate	Effluent anion at perfusion rate (mmol/l)	
				Cl^-	HCO_3^-
cat	2-3 kg	4-9 cm	500 µl/hr	46	110
rat	250-300 g	1.7 - 2.3 cm	100 µl/hr	47	92

Pressure has been implicated in the production of pancreatic duct changes (Simpson 1983, Austin 1984, Pirola 1970) and, because of the previously demonstrated changes of duct extravasation with pressure, this study carefully measured intraduct pressure throughout the experiment. The values obtained at the perfusion rates used were always low, and well within the physiological ranges of the normal pancreatic duct. This experiment required ligation of the bile duct for the study time and a preliminary study on a small ($n=6$) number of animals demonstrated that duct permeability and structure was unaffected by obstruction. Mosley (1981) had also previously noted that long-term jaundice, with high serum bilirubins, failed to alter the characteristics of the pancreatic duct mucosal barrier.

Movement of Cl^- and HCO_3^- ions across the duct wall was investigated and the question of whether anionic movement occurred by an active or passive process determined. Case and Scratcherd (1970) previously showed in cats that Cl^- and HCO_3^- diffused passively through the pancreatic duct epithelium down their respective concentration gradients across the duct wall. Using a similar method this study demonstrated that both anions conformed to Fick's law and moved down their concentration gradients across the duct wall. The duct wall appeared to have a higher permeability for bicarbonate ions than chloride ions. In contrast this permeability is reversed in cats with the feline main pancreatic duct being more

permeable to chloride ions (Case 1970). Greenwell's (1977) suggestion that anions flux through tight junctions between cells rather than through epithelial cells may have significance in relation to our later observations on ultrastructure.

The basal pancreatic secretion over 1 hour was minuscule (4-5 µl/hr), confirming Olazabal's (1983) observations. Cats under basal conditions have no pancreatic secretion (Reber 1980, Case 1970). This may therefore be one disadvantage of the murine model although the very small volumes of pancreatic secretion are almost insignificant in terms of the total volume collected. Indeed the maximum concentration of HCO_3^- (150 mmol/l) secreted by the pancreas over 1 hour in a volume of 5 µl is 0.75 µmol. In contrast 100 µl of perfusate at $[HCO_3^-]$ of 100 mmol/l contains 10 µmol. Thus the maximum contribution that basal pancreatic secretion can make is 7.5% of the total bicarbonate concentration. In reality this contribution is almost certainly much smaller (<5%) making basal secretion unimportant in the context of total HCO_3^- movement.

Perfusion through a biological system requires complete recovery of perfusate since poor recovery invalidates the results. Early studies on the pancreatic duct mucosal barrier failed to take account of volume recovery and relied instead on weighing the effluent fluid (Reber 1979, 1980, Mosley 1981). This method is notoriously insensitive for ensuring completeness of recovery. Olazabal (1983) carefully evaluated recovery in a preparation similar to the present model. He used tritiated inulin as a volume marker and found the percent recovery for the first- and second-hour collections to be 96.1 $\pm$ 1.07% and 94.8 $\pm$ 0.94% respectively.

Simpson (1983) recently described volume recovery in a cat preparation using ^{14}C PEG as a volume marker. He demonstrated that volume recovery was from 92 to 99% and also that a small volume (5-9%) was recovered in the wash solution. I was initially concerned that perfusate might be sequestered in tributaries of the main BPD. Perfusion with ^{14}C PEG demonstrated that at all flow rates the recovery exceeded 94% at 1 hour and only 5% was recovered during the wash period. As the volume of collected effluent was always within $\pm$ 5% of the perfusate volume and the measured basal pancreatic secretion was extremely small I considered that both sequestration and pancreatic secretion had little effect on the ionic concentrations of the effluent measured.

The transductal pD in the rat BPD correlated closely with the difference in $[HCO_3^-]$ between the perfusate and blood. The values obtained were similar to those reported in cats (Mosley 1981, 1979, Moqtaderi 1972) and in rabbits (Reber 1969). The pD's measured were equivalent to the HCO_3^- diffusion potential as the pD was zero when there was no $[HCO_3^-]$ gradient. This is contrary to the situation in the stomach which has a pD of -30 mV (Mosley 1979, Silen 1977).

The normal pancreatic duct of the cat is permeable to bicarbonate and chloride ions (Reber 1979, 1980). Similarly Olazabal (1983) has demonstrated that the normal rat BPD allows some chloride to pass into the duct and some bicarbonate to pass out of the duct. In this study the normal BPD in period I was permeable to both chloride (0.84 μmol/cm/hr) and bicarbonate ions (1.59 μmol/cm/hr). (The increased permeability to bicarbonate ions has been commented on earlier). In control experiments the second hour of collection (period III) gave anionic fluxes almost

indistinguishable from those in the first hour, thus demonstrating both duct stability and the consistancy of duct physiology between animals.

The potential difference across the rat BPD in the control experiments was -2.3 mV, a value close to that observed across the pancreatic duct of cats, -3 to -4 mV (Mosley 1979) and rabbits, -2 to -6 mV (Reber 1969). These results indicate that there is no active secretion by the BPD in contrast to an actively secreting mucosa such as the stomach which has a resting pD of -30 mV (Silen 1977). The pD was very similar between animals and remained stable over the experimental period, reinforcing the concept of duct integrity.

Electron microscopic examination of the duct epithelium is of fundamental importance when considering duct integrity. Although initial research on the pancreatic duct mucosal barrier used relatively insensitive light microscopy (Reber 1979, Olazabal 1983), the importance of ultrastructure has recently become manifest (Simpson 1983). Electron microscopy allows early detection of cell damage and assessment of structures such as tight junctions, intercellular spaces, basal lamina and intracellular organelles. The normal ultrastructure of the main pancreatic duct of the cat has been well described by Reber (1981), Simpson (1983) and Bub (1983) and the importance of the tight junction emphasized by Farquhar (1963) and Ham (1979). The presence studies with rat bile-pancreatic duct mucosa show that the duct cells are unlike gastric surface mucosa cells in that although both have mucus secretory activity, those of the BPD are not developed to the degree seen in the more specialized mucus secreting surface gastric epithelium. The duct cells exhibit predominantly apical adhesion

specializations with relatively loosely adherent basal lateral surfaces, features reminiscent of those of biliary epithelium. The duct cells are columnar with surface microvilli. Tight junctions are present between the apical cell membranes and beneath these is a relatively loose intercellular and basal space. These tight junctions may well have a vital role in maintaining duct integrity. The overall ultrastructural appearance of the rat BPD was very similar to that described in the main pancreatic duct of the cat. Furthermore, the physiological stability of the duct cells was confirmed as at the termination of control experiments the only histological feature was that of minor widening of intercellular spaces.

These initial experiments have enabled us to characterize the physiology of the bile-pancreatic duct of the rat thus.

(i) The ultrastructure of the rat bile-pancreatic duct is similar to that of the pancreatic duct of cat and man.

(ii) Bicarbonate and chloride ions diffuse across the duct wall down concentration gradients. This diffusion is related to the rate of perfusion.

(iii) Volume recovery of perfusate is excellent and basal pancreatic secretion negligible.

(iv) The experimental preparation described is stable over the 4 hour period as evidenced by constant anion flux, pD and an intact ultrastructure.

(v) Perfusion of the rat BPD offers opportunities for studying duct physiology. In particular the effects of varying toxic substances on duct integrity can be evaluated.

CHAPTER IX

THE EFFECT OF BILE, INFECTION AND PRESSURE ON THE PANCREATIC DUCT MUCOSAL BARRIER

Bile alters the permeability of the gastric mucosal barrier (Davenport 1972) and may be important in the pathophysiology of certain diseases such as reflux gastritis and gastric ulceration. This section discusses the effect of bile on the pancreatic duct mucosal barrier (PMB) and in a later chapter the various individual biliary constituents (e.g. bile salts, lysolecithin) are evaluated further.

Reflux of bile or duodenal juice into the pancreas may be the initial step in the pathogenesis of acute gallstone pancreatitis. As common bile duct contents can occasionally reflux into the pancreatic ducts without producing untoward effects (Taylor 1980, Ivy 1952), it appears that both the nature of and pressure of refluxed bile are important. Konok and Thompson (1969) postulated a barrier between duct contents and the pancreatic parenchyma and a defect in this pancreatic duct mucosal barrier was proposed as the earliest step in the evolution of acute gallstone pancreatitis.

Konok and Thompson (1969) studied the effects of various substances on the integrity of the feline pancreatic duct. They used low pressure infusion and measured duct integrity by light microscopy and by the absorption of curare from the damaged duct which produced animal death. Sterile bile and E. coli suspension had no effect on the duct mucosa; bile incubated with pancreatic juice caused moderate damage to the epithelium. The most obvious and spectacular changes occurred after

infected (E. coli) bile was infused when immediate and progressive destruction of the duct wall resulted. Bile infected with E. coli thus showed mucolytic and cytotoxic effects far beyond those of the other solutions tested. In all cases the curare test was positive. Their conclusions were that at low infusion pressures the duct was remarkably resistant to sterile bile, activated pancreatic enzymes and bacteria. Infected bile was the most potent factor in breaching this barrier. Unfortunately Konok and Thompson did not further pursue these interesting results.

Mizumoto and associates (1971) postulated that the protective pancreatic barrier might be due to mucopolysaccharides present in the thin mucous layer. They thus investigated the effects of beta glucuronidase, produced by E. coli bacteria, on the duct mucosa. Beta glucuronidase alone produced a reduction in the mucous layer. Sterile bile alone produced no untoward effects. However, when bile was mixed with beta glucuronidase there was evidence of duct destruction with associated severe necrotizing pancreatitis. The results of this study suggested therefore that beta glucuronidase produced by bacteria might be the reason for the extreme toxicity of infected bile.

Reber and colleagues (Reber 1979, 1980, Mosley 1981) characterised the pancreatic duct mucosal barrier fully, and measured PMB stability by means of anion flux. In their studies sterile bile did not increase the permeability of the duct, but perfusion with infected bile did. It is noteworthy that the effect of infection was seen only when the bacteria in the perfusate were pathogens known to infect bile and not when urinary pathogens were used. They attributed this difference to differing abilities

of the various bacterial species to convert the primary bile acids to secondary bile acids (i.e. deoxycholate and lithocholate) (Carey 1973). Moreover, Reber suggested that deconjugation of bile acids by bacteria might also contribute to the toxicity of infected bile, and that bacteria disrupted bile micelles and increased the effective concentration of bile salts in solution. The explanation for infected bile toxicity is further complicated by the fact that bacteria convert virtually all the biliary lecithin into lysolecithin (see later). Thus the efficacy of infected bile in damaging duct integrity may be because of:

(i) Bacteria produce toxins in bile, e.g. beta glucuronidase.

(ii) Bacteria convert primary bile acids into secondary bile acids which are known to be toxic.

(iii) Bacteria deconjugate bile acids into their toxic unconjugated derivatives.

(iv) Bacteria disrupt micelles and thus increase the availability of bile acids in bile.

(v) Bacteria convert harmless lecithin into toxic lysolecithin.

There is a need both to substantiate these findings and to evaluate the ultrastructural changes induced by bile. In addition correlation of anion flux, potential difference and ultrastructure should be investigated in order to fully assess pancreatic duct integrity.

The nature of bile in patients with gallstones and acute pancreatitis has received little experimental attention. Heuman and co-workers (1980) studied the gall bladder bile composition in patients with cholesterol gallstones. They determined that bile from patients with gallstones

contained relatively more deoxycholates and less lecithin than did bile from patients without gallstones. Since deoxycholates are more effective at producing inflammation than cholates and lecithin is possibly protective (see later) these findings might be of relevance in considering bile induced damage to the pancreatic duct epithelium. There is, however, a paucity of knowledge concerning (i) the chemical makeup of "pancreatitic" bile and (ii) its effects on pancreatic duct integrity compared to ordinary sterile bile.

Although the effects of various substances on the PMB have been evaluated little attention has been given to the effects of pressure. Mosley (1981) emphasized that the perfusion pressured used in his experiments was always low (i.e. less than 8 cm H_2O). Recently, Simpson and co-workers (1980, 1983) have evaluated the effects of pressure on the pancreatic duct mucosal barrier. They studied both low (3.7 cm H_2O) and high (30 cm H_2O) pressures, both alone and in conjunction with a bile salt. High pressure alone did not significantly alter anionic flux and electron microscopy showed minor inconsistent alterations of cell structure. When however pressure was combined with taurocholate there was a marked increase in permeability and notable ultrastructural changes resulted. These results indicate that pressure alone is non-damaging to the PMB but when combined with a toxic intraluminal substance then there is a marked increase in "barrier" damage. Austin and Sieckman (1984) recently reported on pancreatic duct permeability to macromolecules. They found that increased pancreatic duct pressure, achieved with secretin stimulation and duct obstruction, increased pancreatic duct permeability to molecules the size of pancreatic duct enzymes (mol. wt. 17,500).

These observations can be summarized thus:

(1) Sterile bile has limited toxicity to the pancreatic duct.

(2) Infected bile is extremely damaging to pancreatic duct epithelium.

(3) High pressure may increase bile toxicity.

(4) The effect of "pancreatitic" bile on the pancreatic duct is unknown.

Materials and Methods

Object:

To evaluate the effect of "bile", infection and pressure on pancreatic duct integrity.

Experimental preparation

This was as described in the preceeding chapter (fig. 28). Careful attention was given to the control of pressure by means of the pressure transducer in the perfusate cannula. Five animals were studied at each pressure in all of the five experimental groups. The experimental sequence was thus;

period I - SPS perfusion x 1 hour.

period II - "Bile" perfusion at low or high pressure x 20 mins.

period III - SPS perfusion x 1 hour.

Solutions:

The solutions were as described in chapter V. Briefly these solutions were;

(i) SPS - standard perfusate solution i.e. control.

(ii) SPS + E. coli - a fresh bacterial suspension of biliary Escherichia coli organisms; 10^5 - 10^6 orgs/ml.

(iii) sterile bile - sterile choledochal bile taken from 10 patients.

(iv) "pancreatitic" bile - sterile choledochal bile from 4 patients with a recent history of acute gallstone pancreatitis.

(v) infected bile - infected T-tube bile taken from 8 patients.

Bile solutions from different patients gave similar results, i.e. sterile bile from all the 10 patients gave comparable results as did those of "pancreatitic" and infected bile.

Pressure:

The pressure was carefully measured by means of a Bell and Howell (type 4/422) pressure transducer incorporated via a 3-way tap into the infusion system. Pressure in the system could be altered by raising or lowering the height of the effluent cannula until the values required were registered on the recorder (Simpson 1983). The pressures studied were

low pressure - 8 cm H_2O
high pressure - 35 cm H_2O
} (100 µl/hr)

PMB damage

Damage to the PMB or duct integrity was assessed as previously described.

(a) Anionic flux of Cl^- and HCO_3^- Mean changes in ion flux between periods III and I were measured.
In controls with a stable duct the change is very small. Increased damage to duct integrity is represented by increasingly large mean changes in ion flux.

(b) Transductal potential difference (pD) change from periods III to I. Increased duct damage resulted in an increased pD change.

(c) ultrastructual examination of the duct was performed at the end of the experiment as previously described.

Results (figures 33A-C; tables 23,24)

Controls:

The experimental preparation was stable throughout the procedure. At low pressures the anionic flux and pD remained stable over the two periods (table 23). This stability of the duct was emphasized by the normal electron microscopy (fig. 34A). High pressure infusion produced minor increases in chloride flux and pD (table 24). Although these differences were just significant bicarbonate flux remained constant indicating only a slight increase in duct permeability. Electron microscopy demonstrated widened intercellular spaces (fig. 34B). The tight junctions and cells themselves were normal.

E. coli solution

Infusion of a bacterial suspension at low pressure produced no change in duct permeability as assessed by ion flux, pD and ultrastructure (table 23). High pressure produced slight increases in ion flux of Cl^- and HCO_3^- (table 24), differences that were just significant (figs. 33A-C). The electron microscopic appearances were virtually identical to those of SPS alone with widened intercellular spaces being the only abnormality. As E. coli solution behaves similarly to SPS alone, it appears that bacteria themselves have no effect on duct integrity.

Sterile bile

At low pressures sterile bile produced moderate increases in anionic flux ($P<0.01$) and a reduction in pD ($P<0.01$) (table 23). The ultrastructural appearance was that of swollen and oedematous duct cells. There was no evidence of epithelial disruption or damage to

the junctional complexes (fig. 34C). At high pressures (table 24) these changes were markedly increased compared with the corresponding experiment at low pressure ($P<0.001$). Anionic flux increased two to three fold and pD changed considerably. Electron microscopy demonstrated swollen epithelial cells with large basal and intercellular spaces. The luminal surface of the cells had reduced microvilli (fig. 34D).

"Pancreatitic" bile:

At low pressure anionic flux of Cl^- ($P<0.001$), HCO_3^- ($P<0.01$) and pD ($P<0.001$) were significantly different to those seen with sterile bile (table 23). On electron microscopy the epithelial cells were swollen with occasional vacuolation, and the intercellular spaces were widened. Occasional areas of epithelial disruption were apparent. At high pressure anionic flux was significantly ($P<0.01$) increased over the corresponding low pressure values, although pD remained fairly constant. Although the ion flux of chloride was higher than for sterile bile, bicarbonate flux and pD change were of similar magnitude (table 24). The ultrastructural appearances at high pressure were those of swollen cells, wide basal spaces and several areas of cell shedding. These changes were more marked than with low pressure or when sterile bile was infused at high pressure.

Infected bile:

At both pressures infected bile produced significantly more damage to the bile-pancreatic duct than the other solutions. At low pressure both anionic flux ($P< 0.001$) and pD ($P< 0.05$) were significantly different than after perfusion with the other "bile" solutions (table 23). On electron microscopy there was evidence of pronounced epithelial disruption

(fig. 34E) with cell shedding, damage to the tight junctions and exposure of the basement lamina. High pressure increased these ultrastructural changes (table 24). There was a significant increase in ion flux ($P<0.01$) and pD ($P<0.01$) over the low pressure values (fig. 33A-C). The ultrastructural appearance was that of severe duct damage. Virtually all the epithelial cells had been stripped from the duct wall and there was collection of cell debris in the lumen (fig. 34F). The basement lamina was exposed in most places and in several areas there was evidence of disruption of the lamina with subjacent tissue oedema (figs. 34G+H).

Results summary (changes 0 to +++)

	Low pressure	High pressure
SPS	flux/pD 0. ultrastructure normal	0/+ widened intercellular spaces
SPS + E. coli	flux/pD 0. ultrastructure normal	0 widened intercellular spaces
Sterile bile	flux/pD + swollen cells	++ swollen cells, wide intercellular spaces
"Pancreatitic" bile	flux/pD ++ cells swollen/ vacuolated	++ wide basal spaces cell shedding
Infected bile	flux/pD +++ epithelial disruption	+++ luminal debris damaged basement lamina

(0 normal, +++ marked change)

The results of bile, infection and pressure on the bile-pancreatic duct can be summarized as:

1. Bacterial solution of E Coli produces no damage.

2. Sterile bile produced moderate damage which is increased by pressure.
3. "Pancreatitic" bile is more toxic than sterile bile.
4. Infected bile produces by far the worst damage to the duct epithelium. Ion flux and pD changes are maximal at high pressure.

TABLE 23 Bile + infection v. Anionic Flux (mean ± SD)
(low pressure)

	mean change in net flux (μmol/cm/hr)		change in pD (mv)
(No)	Δ J.Cl^-	Δ J.HCO_3^-	Δ pD
SPS (5)	+0.10±0.01	−0.07±0.01	−0.1±0.01
SPS + E. Coli (5)	−0.02+0.01	−0.06±0.01	+0.1+0.02
Sterile Bile (5)	+0.43±0.08*	−0.33±0.06*	+0.21±0.04*
Pancreatitic Bile (5)	+0.84±0.13+	−0.45±0.05‡	+0.55+0.07+
Infected bile (5)	+1.29±0.15§	−1.30±0.14§	+0.60±0.04Π

Sterile v. control *$P<0.01$

Pancreatitic v. sterile +$P<0.001$, ‡$P<0.01$

Infected v. all §$P<0.001$, Π$P<0.05$

TABLE 24 Bile +infection v. Anionic Flux (mean ± SD)
(high pressure)

	mean change in net flux (μmol/cm/hr)		change in pD (mv)
(No)	$\Delta J.Cl^-$	$\Delta J.HCO_3^-$	Δ pD
SPS (5)	+0.14±0.02*	−0.10±0.02	+0.15±0.02*
SPS + E. Coli (5)	+0.03±0.01+	+0.03+0.01+	+0.1±0.01
Sterile Bile (5)	+1.12±0.10‡	−0.77±0.05‡	+0.61±0.04‡
Pancreatitic Bile (5)	+1.47+0.14Π§	−0.80±0.09Π	+0.57±0.01
Infected bile (5)	+1.98±0.17¶	−1.45±0.12¶	+1.05±0.13¶

SPS v. low pressure *P<0.05

SPS + E. Coli v. low pressure +P<0.02

Sterile v. control ‡P<0.001, v. low press. ‡P<0.001

Pancreatitic v. sterile §P<0.01, v. low press. ΠP<0.01

Infected v. all ¶P<0.001, v. low press. ¶P<0.01

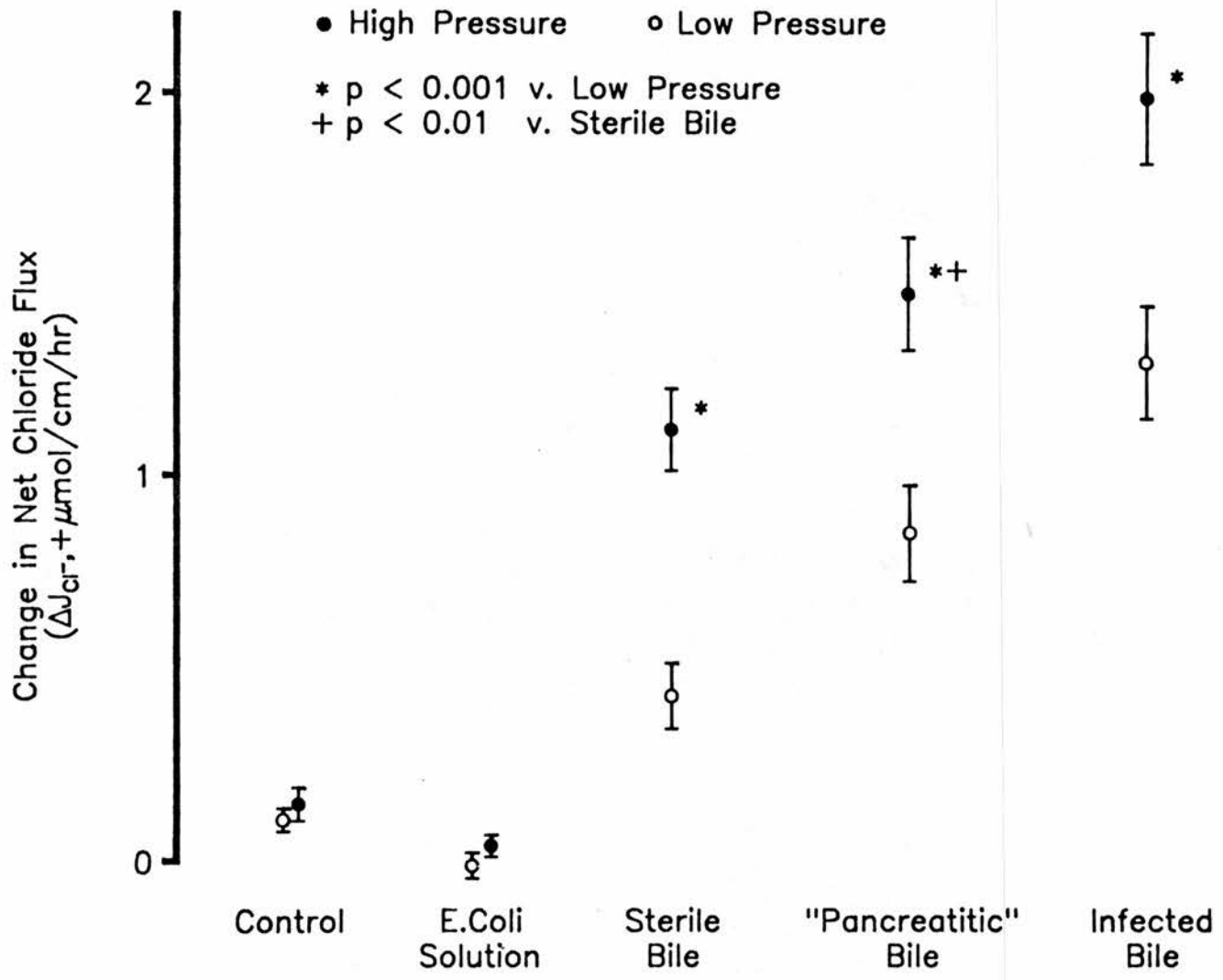


Fig. 33A "Bile" infection and pressure vs. chloride flux.

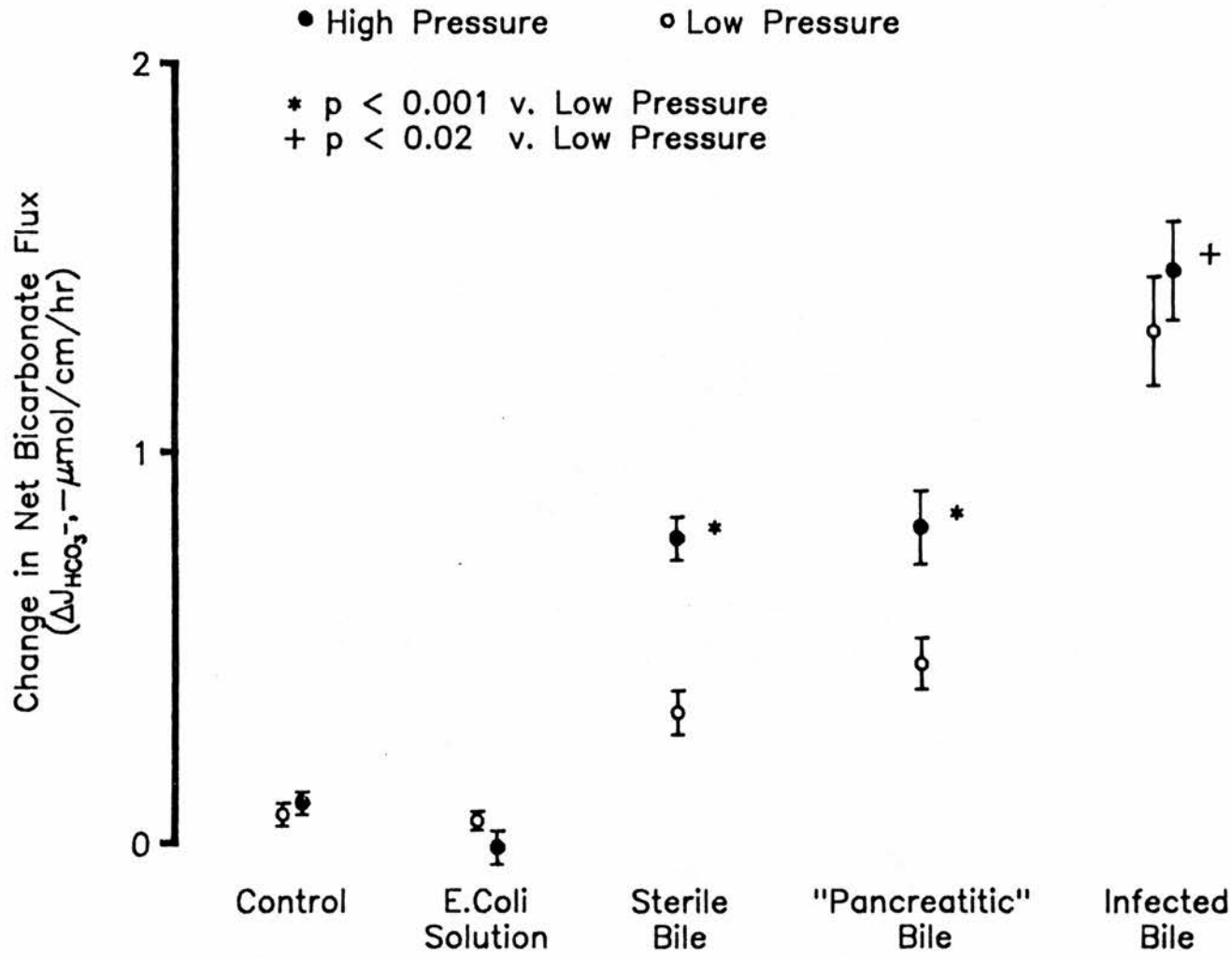


Fig. 33B "Bile", infection and pressure vs. Bicarbonate flux.

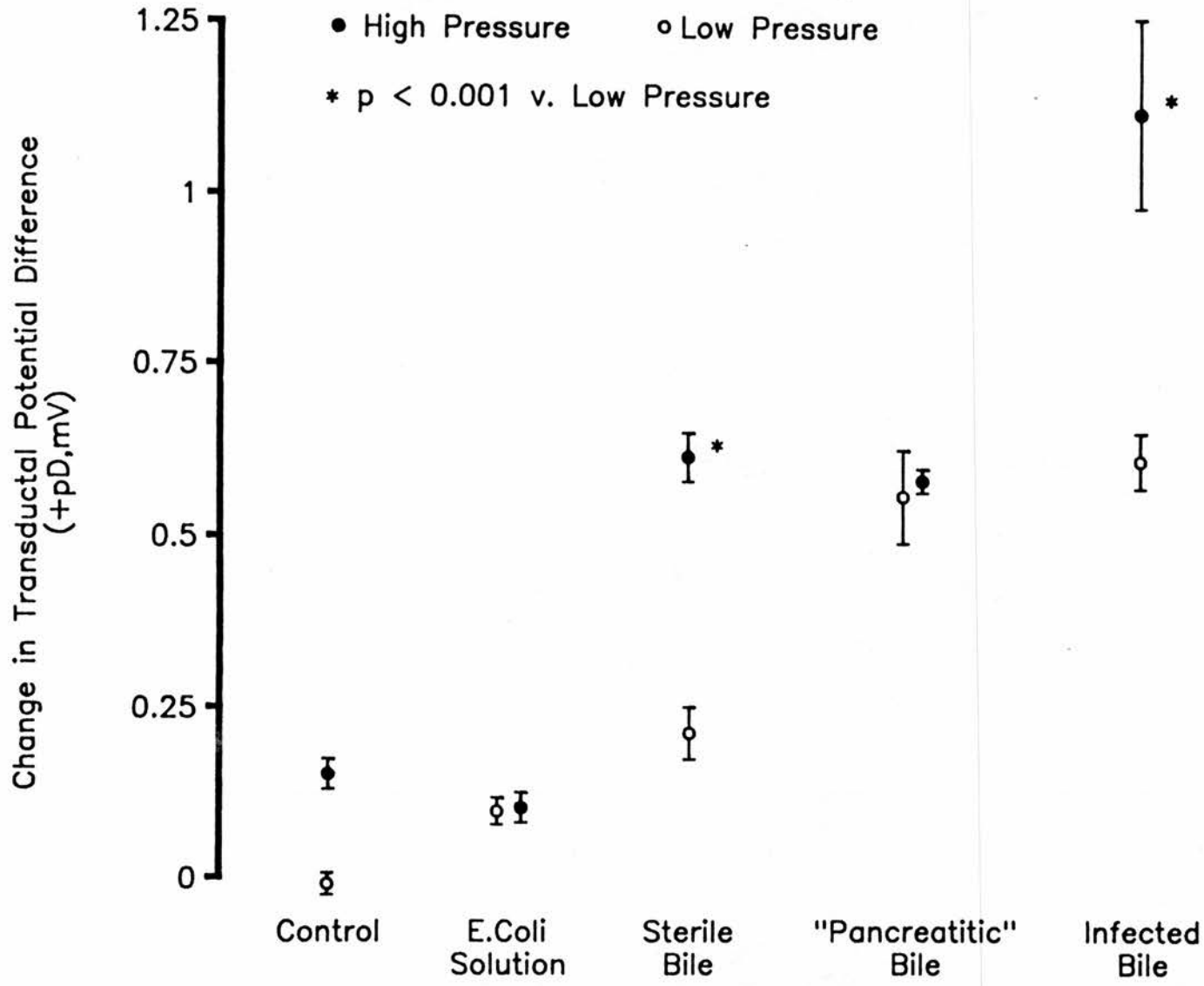


Fig. 33C "Bile", infection and pressure vs. pD.

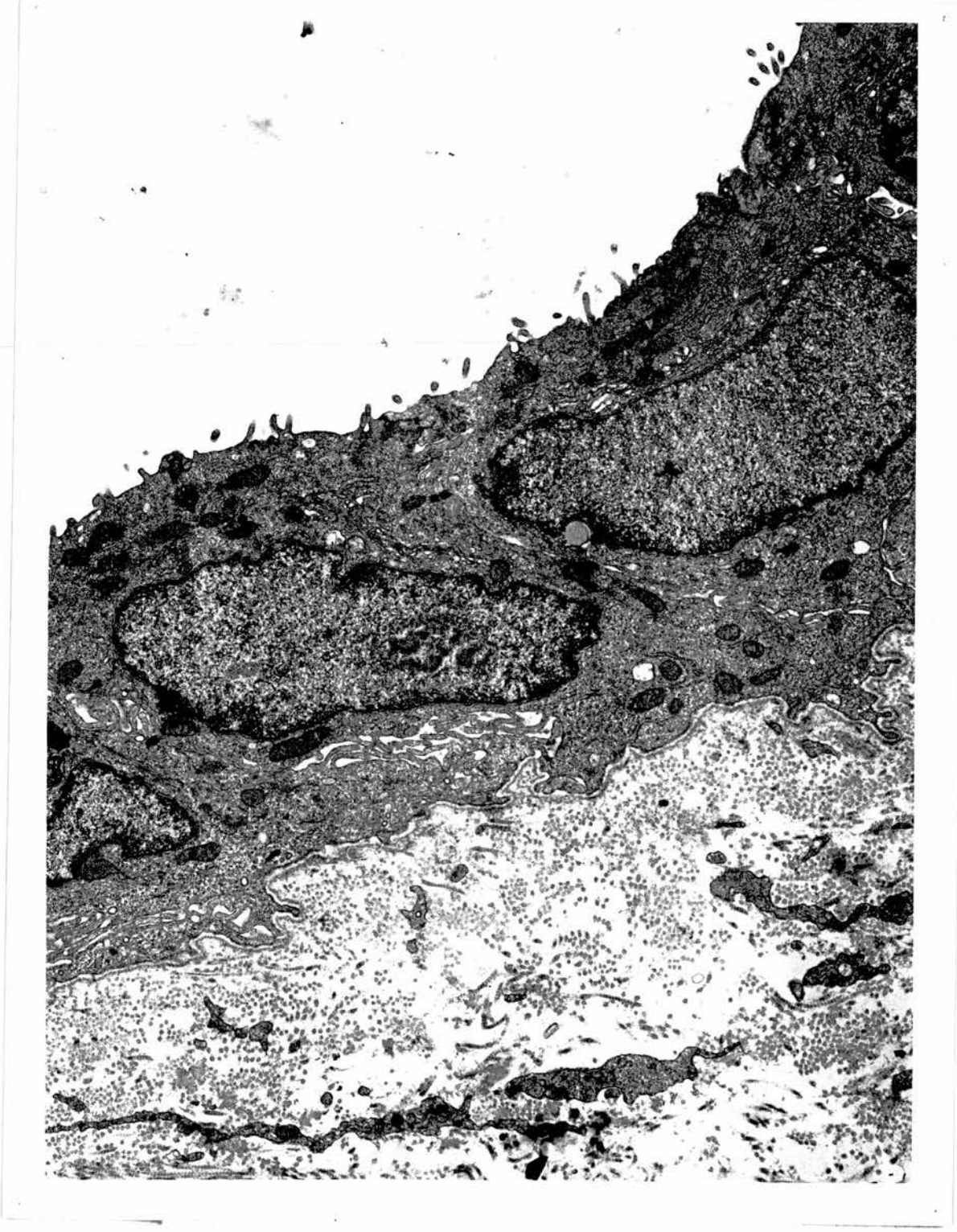


Fig. 34A Electron microscopy after perfusion of duct with SPS at low pressure (x 11250). (see p. 168)

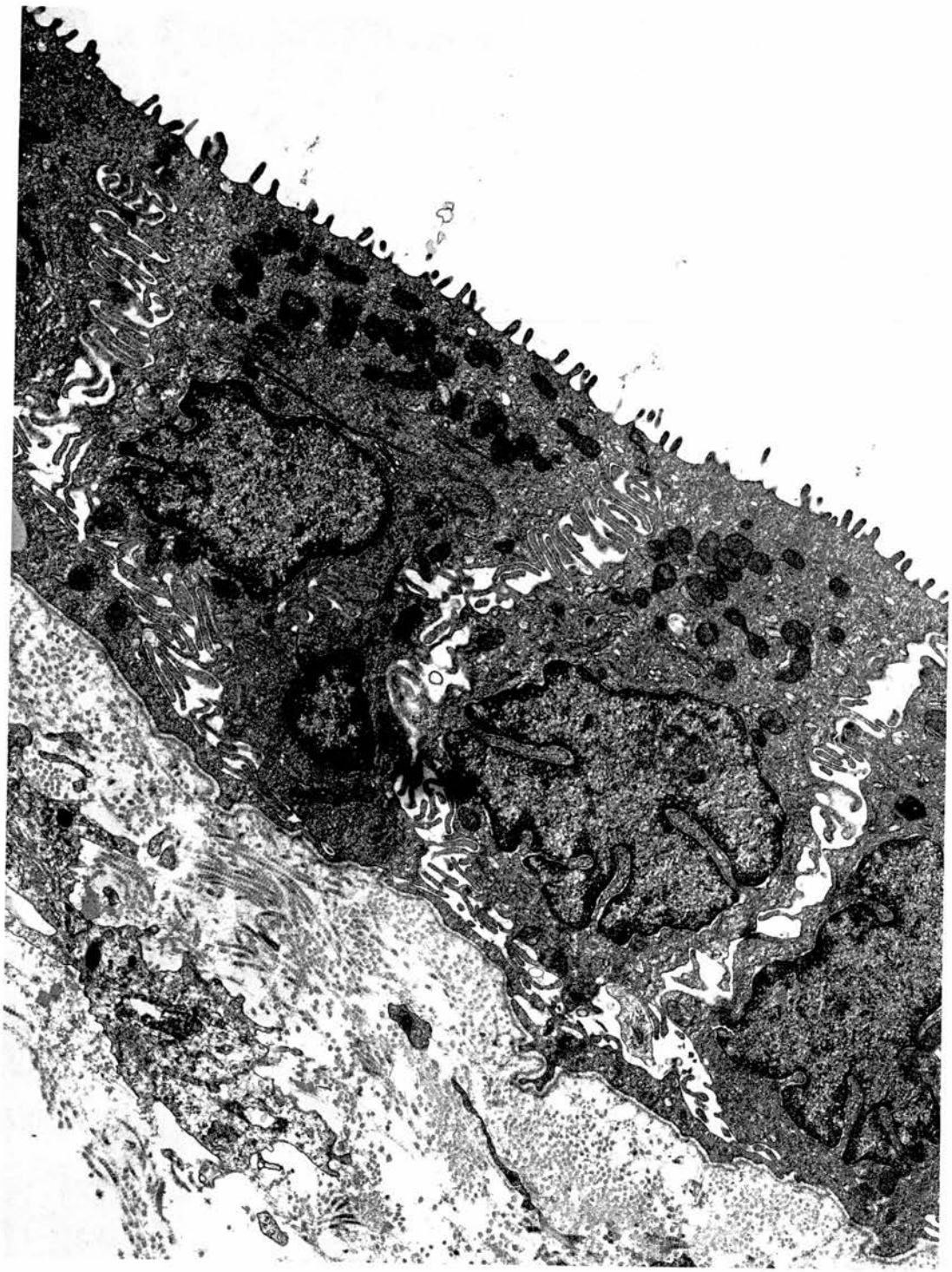


Fig. 34B Electron microscopy after perfusion of duct With SPS at high pressure (x 11250). (see p168)

Fig. 34C Electron microscopy after perfusion of duct with sterile bile at low pressure (x 7500). (see pp 168-9)

Fig. 34D Electron microscopy after perfusion of duct with sterile bile at high pressure (x 11250).

Note grossly widened intercellular spaces. (see p 169)

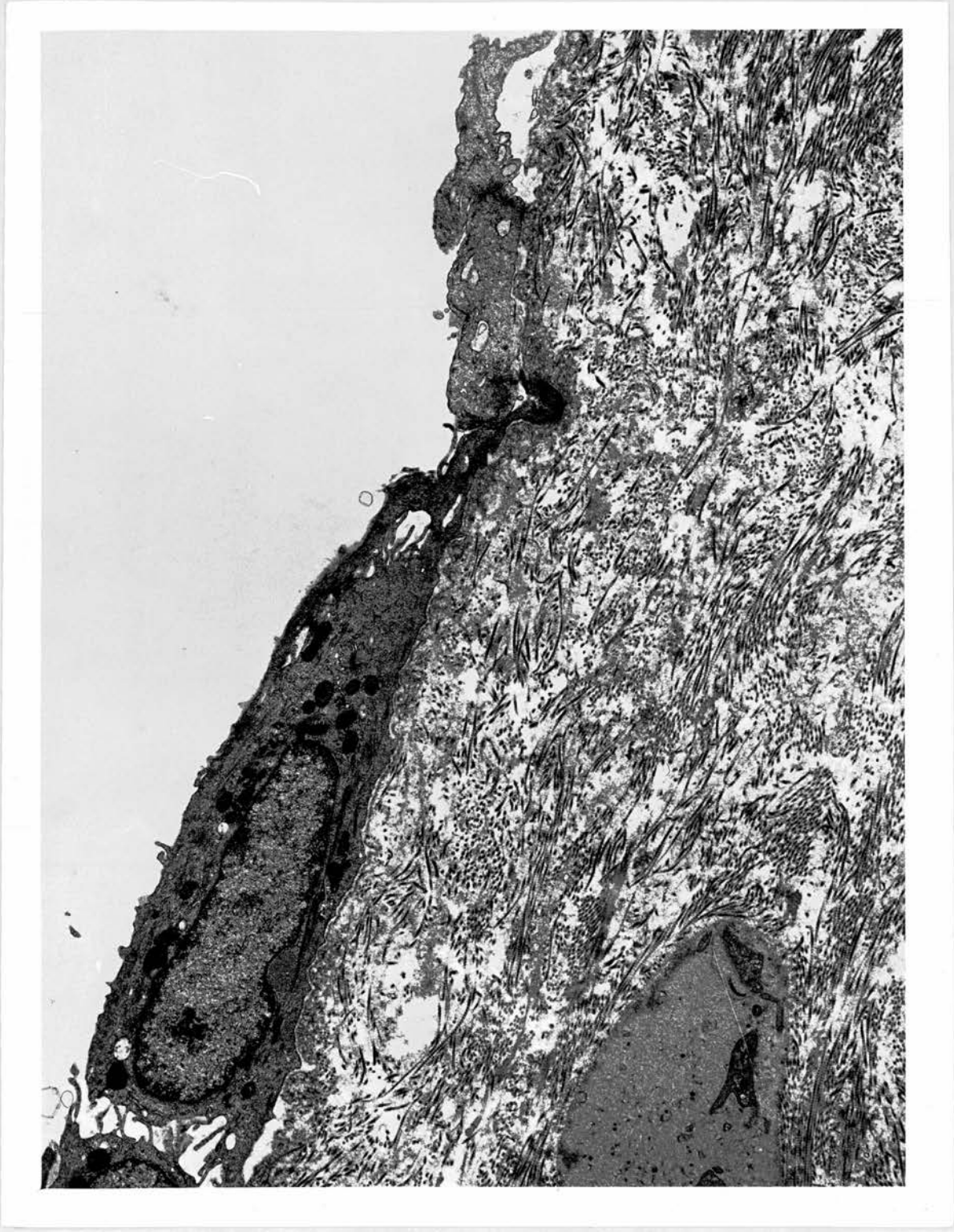


Fig. 34E Electron microscopy after perfusion of duct with infected bile at low pressure (x 7500).

Note widened intercellular spaces and cell death. (see p169-70)

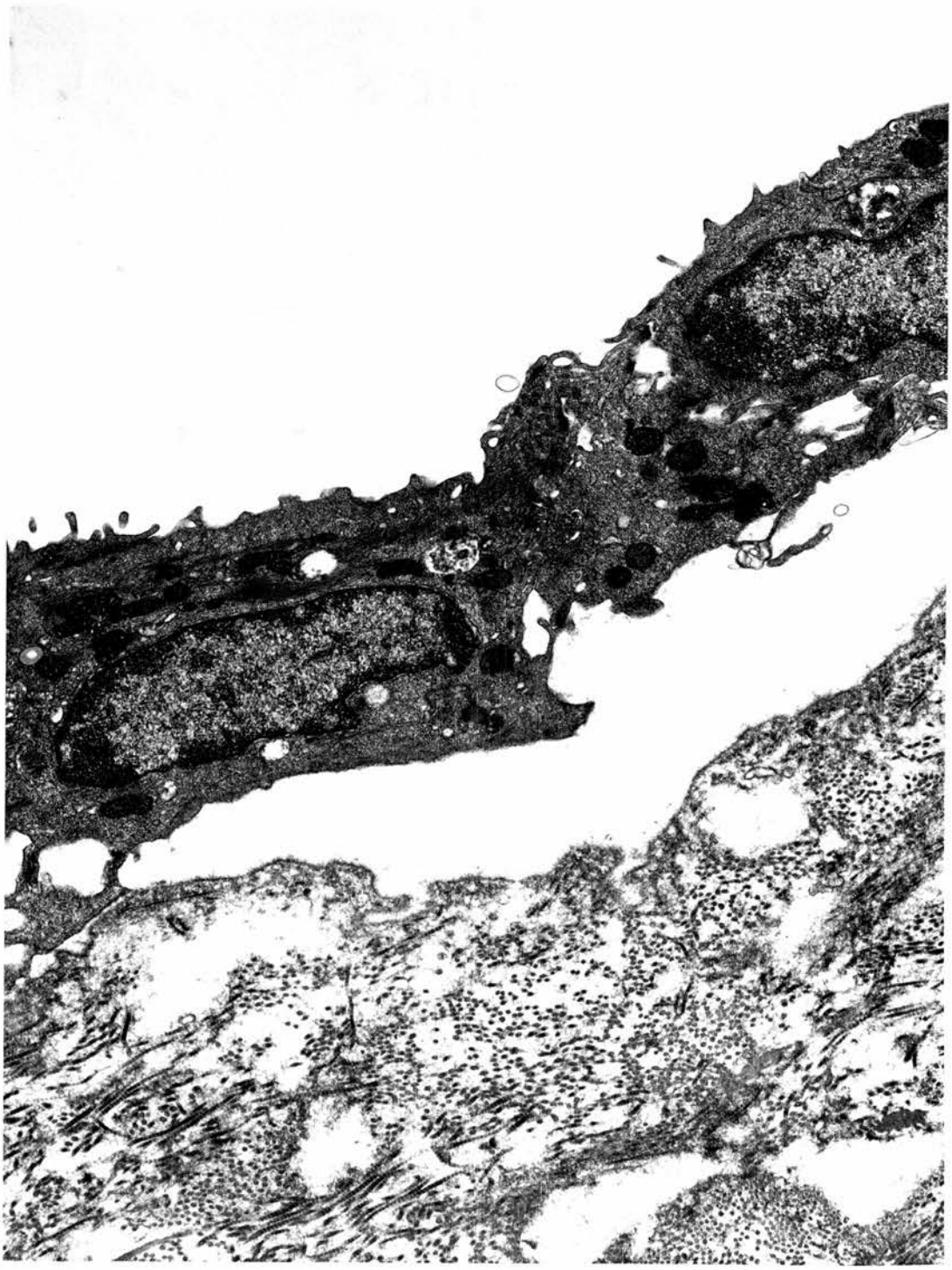


Fig. 34F Electron microscopy after perfusion with infected bile at high pressure (x 11250).

Note epithelium lifted from basement lamina. (see p 170)

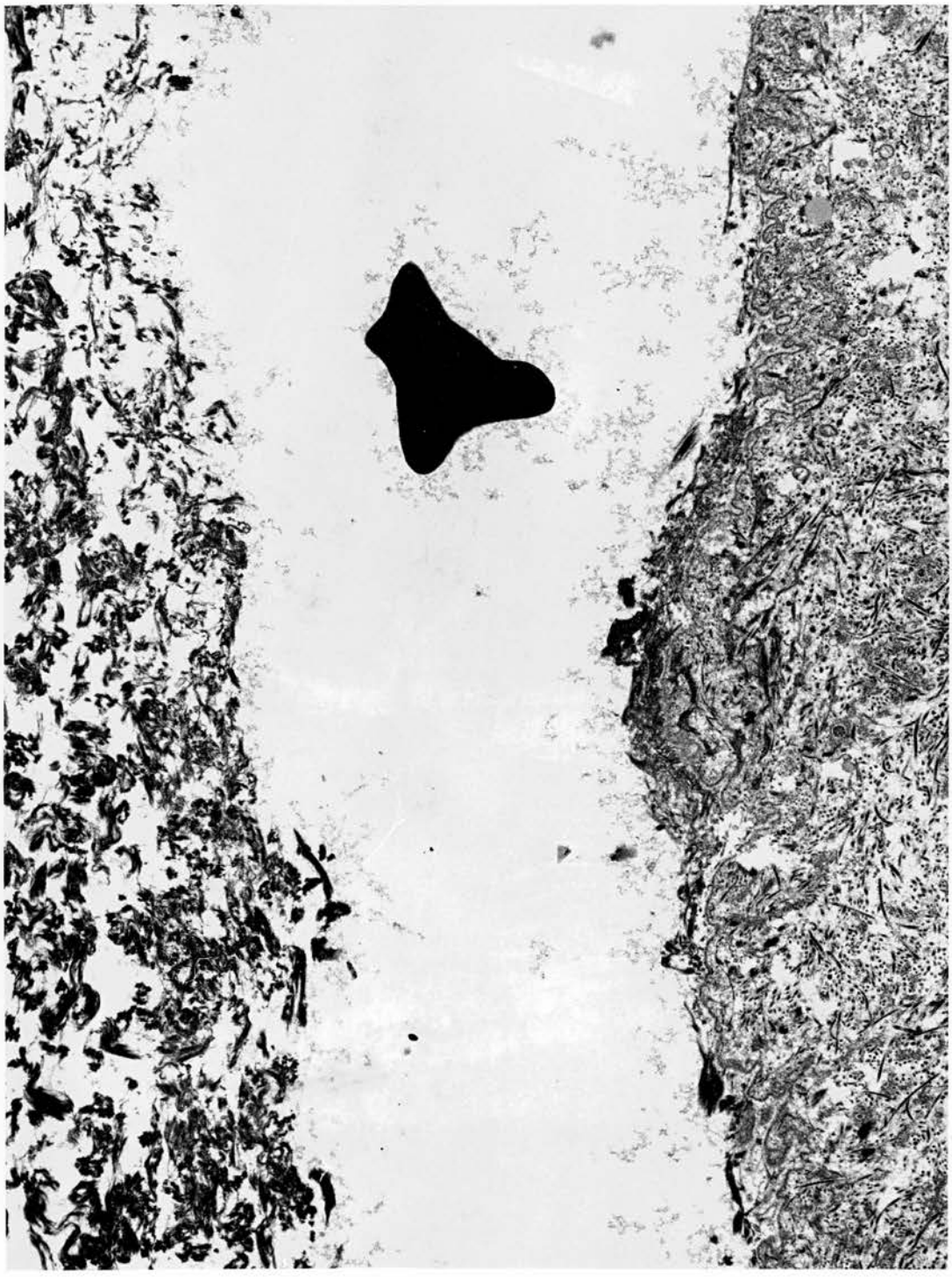


Fig. 34G Electron microscopy after perfusion of duct with infected bile (x 7500).

Note complete epithelial loss. Debris and erythrocyte in lumen. Basement lamina damaged in places. (see p170)

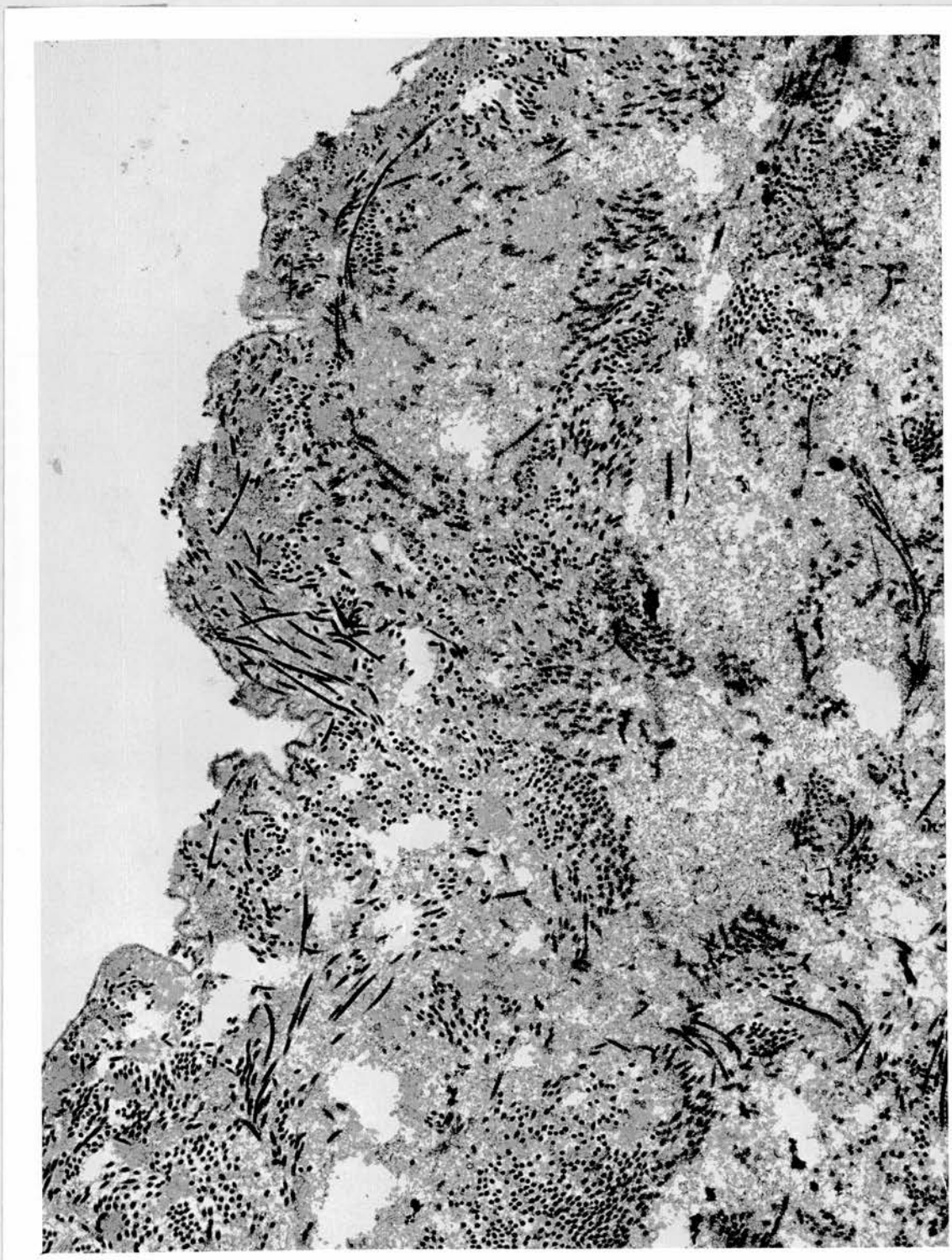


Fig. 34H Electron microscopy after perfusion of duct with infected bile (x 11250).

Note complete epithelial loss, disruption of basement lamina and subjacent oedema. (see p 170)

Discussion:

These experiments have demonstrated the effectiveness of the murine in situ model of BPD perfusion in investigating duct physiology. Following the earlier observations (chapter V) on bile and the production of pancreatitis, I was intrigued to evaluate the effects of the varying types of bile on duct integrity. The earlier observations had demonstrated that differing types of bile had varied pancreatotoxicity i.e. infected>"pancreatitic">sterile and that high pressure was more noxious than low pressure.

Perfusion with the standard solution at high pressure produced minor changes in duct permeability. The only ultrastructural abnormality was a widening of intercellular spaces with the cells and tight junctions being normal. These results are similar to the only other comparable experiment of Simpson et al (1983) as regards permeability changes, but Simpson could demonstrate no alteration of the intercellular spaces. Pressure itself, therefore, produces no important change in duct integrity.

A fresh bacterial solution, containing proven viable organisms, produced no significant damage to the PMB. In addition, high pressure did not increase permeability to any great degree and the only microscopic changes were those of widening of the intercellular spaces. It appears that bacteria themselves are non-toxic to the pancreatic ducts, confirming the observations of Konok and Thompson (1969).

Perfusion with sterile bile produced moderate damage to the PMB and swollen duct cells were seen on electron microscopy. These changes were

increased by high pressure when there was evidence of loss of microvilli and an increase in the size of the basal and intercellular spaces. Widening of intercellular and basal spaces appears to be one of the earliest responses of the duct epithelium after perfusion with toxic substances (Simpson 1993). The importance of pressure in producing damage to the duct epithelium was demonstrated as high pressure increased anion flux two- to three- fold and changed the pD considerably. Previous authors (Konok 1969, Mizumoto 1971) have commented on the lack of toxicity of sterile bile to the duct mucosa. Later studies by Reber (1979, 1980) and Mosley (1981) demonstrated sterile bile to have little effect on duct permeability. It is important to emphasize that all these researchers used only low pressure and did not study mucosal ultra-structure. Indeed, in our study the most marked alterations in duct integrity occurred at high pressures. The only comparable study using careful pressure measurements was that of Simpson (1983) who demonstrated that high pressure plus bile salts increased ion flux two-fold and produced a marked widening of basal spaces and epithelial disruption; results similar to those reported in this study. Sterile bile produces mild to moderate damage to the PMB and this damage is markedly increased by high pressures.

I have previously demonstrated that "pancreatitic" bile is more toxic than sterile bile, and have postulated some change in its chemical makeup. The recent observations of Braganza and colleagues (1983a,b) suggest that altered bile is responsible for pancreatic disease. The results of this study confirm my earlier results on the toxicity of "pancreatitic" bile. At low pressure, "pancreatitic" bile produced significantly more damage to the PMB than sterile bile; as ion fluxes

were much greater, the pD reduced considerably and duct cells demonstrated oedema and vacuolation. High pressure increased ion flux, although to a much less degree than with sterile bile, and the occasional areas of cell shedding on electron microscopy attested to its increased toxicity. Thus "pancreatitic" bile, even at low pressures, is very damaging to the ducts. These findings are new as no previous investigator has studied bile from patients with acute gallstone pancreatitis.

Infected bile was shown by earlier investigators to produce more severe damage to duct epithelium than sterile bile (Konok 1969, Mizumoto 1971, Reber 1979, 1980, Mosley 1981). Whether this is due to bacteria acting on bile salts or on other bile constituents such as lysolecithin remains uncertain. The earlier results after perfusion in the bacterial solution do, however, suggest that the bacteria themselves are relatively non-toxic to the pancreatic duct. In this study infected bile produced the most marked increases in duct permeability and these changes, although noticeable at low pressures, were most apparent with high pressure infusion. Indeed the magnitude of ion flux was such as to suggest complete destruction of the pancreatic duct mucosal barrier. Electron microscopy demonstrated almost complete loss of the duct epithelium and exposure of the basement lamina. Moreover in some places the basement lamina was damaged with subjacent oedema of the connective tissue. It is not difficult to imagine toxic substances leaking from such damaged ducts and initiating pancreatic inflammation and these results reinforce our previous observations on the toxicity of infected bile.

Several conclusions can be made from this study.

1. High pressure alone widens intercellular spaces but does not appreciably increase duct permeability.
2. Bacteria in solution are non-toxic to the ducts.
3. Sterile bile produces moderate damage to the duct integrity; this damage is markedly increased by high pressure.
4. "Pancreatitic" bile, especially at low pressure, damages the PMB to a much greater degree than sterile bile. This suggests that the nature of bile itself may be altered in patients with acute gallstone pancreatitis.
5. Infected bile destroys the pancreatic duct mucosal barrier at both high and low pressures.
6. Although pressure alone is unimportant, when combined with a toxic substance such as bile it becomes significant.
7. Measurement of one aspect of "barrier" function is an insensitive method of determining damage. Using four aspects, as in this study, enables a more critical evaluation of duct integrity.

CHAPTER X

THE EFFECT OF BILE SALTS ON THE PANCREATIC DUCT MUCOSAL BARRIER

Bile salts are one of the most powerful naturally occurring detergents (Martin 1981, Helenius 1975), and have been shown to increase the permeability of biological membranes. Reflux of bile acids into the stomach has been implicated in the initiation of gastritis and gastric ulceration by altering the permeability of the gastric mucosal barrier (Reber 1980, Davenport 1972). Bile salts are assumed to disrupt surface epithelial cells by dissolution of membrane lipids (Morris 1984, Duane 1980) and the application of bile salts to epithelial surfaces produces damage within minutes (Morris 1984). Furthermore, these naturally occurring surfactants have been also shown to break down mucus structure (Martin 1981, 1978) and to be directly toxic to membranes (Kellaway 1977).

It is well recognised that bile salts increase the permeability of gastric mucosa to H^+ ions and promote ulcergenesis (Simpson 1983, Black 1971). These agents may also cause severe damage to surface cells when luminal pH is held at values where virtually no H^+ enters the tissue. Forte and associates (1976) examined the effect of deoxycholate on frog gastric mucosa when luminal pH was maintained above the pKa of deoxycholate (pKa = 6.3) to prevent bile salt precipitation. Ultrastructural changes observed included swelling of the entire apical region of the epithelial cells, loss of microvilli, and in extreme cases, complete cell disintegration so that only remnants of cytoplasmic organelles remained. However, the junctional complexes between the cells appeared resistant to disruption and retained their

structural integrity despite cell disintegration. These observations on gastric surface epithelium may well have relevance in the context of pancreatic duct integrity.

Reber and colleagues (1979, 1980) examined the effect of various bile salts on the feline pancreatic duct mucosal barrier. They demonstrated that pancreatic duct permeability was increased by exposure to various bile salts at a range of physiological concentrations. Moreover, secondary bile acids (deoxycholate) were more damaging than primary acids (cholate). Significant differences between the effects of conjugated and unconjugated bile salts on duct permeability at an alkaline pH were found. In contrast, the tauro- and glyco- conjugates appeared equipotent. Increasing concentrations of bile salts produced a greater change in duct permeability. Perfusion of the duct with mixtures of bile salts produced additive effects on membrane permeability. Histological assessment of the duct system was unremarkable regardless of the changes in duct permeability although ultrastructural examination was unfortunately not performed. The results of Reber's experiments can be summarized as; secondary bile salts are more toxic than the primary salts, dihydroxy bile salts are more toxic than the trihydroxy derivatives, glyco- and tauro- conjugates are equally toxic , unconjugated bile salts are more toxic than the conjugated derivatives, and an increased concentration of bile salts produce increased damage to the pancreatic duct.

The various bile salts have differing effects on pancreatic duct permeability and can be roughly graded as; glycodeoxycholate, taurodeoxycholate, and deoxycholate are more toxic than chenodeoxycholate

derivatives which in turn are more toxic than cholic acid salts.

Simpson (1983) and Reber (1981a) examined the effect of bile salts on mucosal ultrastructure. Both glycodeoxycholate (Reber 1981a) and taurocholate (Simpson 1983) produced marked electron microscopic changes in the duct cells. These were widening of the intercellular spaces, loss of surface microvilli and occasional cell degeneration. Interestingly the junctional complexes were normal, analogous to the gastric mucosal epithelium (Forte 1976). These changes in the duct epithelium were accompanied by marked alterations in duct permeability. Ducts with the most marked mucosal disruption had the highest permeability. As previously described, Simpson (1983) demonstrated that these bile salt changes could be increased by pressure.

Olazabal (1983) evaluated the effect of unconjugated deoxycholic acid on the rat bile-pancreatic duct. He showed that 5 and 10 mM solutions of deoxycholate increased the duct permeability to Cl^- and HCO_3^- ions; in contrast lower concentrations of bile salt had little effect. These increases in duct permeability were accompanied by severe duct epithelial necrosis on light microscopy.

Although bile salts were shown to increase duct permeability to Cl^- and HCO_3^- anions it was uncertain until recently as to the relevance of this observation as regards the aetiology of acute gallstone pancreatitis. Reber (1981b, 1982) and other workers (Wedgewood 1984, Austin 1984) have demonstrated that 15 mM glycodeoxycholate increases duct permeability to macromolecules of molecular weight 20 - 40,000. Since the enzymes which have been implicated in the pathogenesis of acute pancreatitis are of similar

size (e.g. trypsin 25,000, phospholipase A_2 14,800) the pancreatic duct exposed to bile salts is probably permeable to them as well. Such diffusion of enzymes out of the duct into the parenchyma after bile salt damage may be important in the pathogenesis of gallstone pancreatitis. O'Leary and colleagues (1982) further demonstrated an increased permeability of the pancreatic duct to macromolecules after bile salt damage.

Interestingly it has recently been shown (O'Leary 1984) that deoxycholate produces hyperplasia of pancreatic duct epithelium as well as increasing the duct permeability. These results suggest that secondary bile acids or other similar surface-active cytotoxic agents present in the biliary tree or duodenum may play an important role in the pathogenesis of pancreatic duct epithelial hyperplasia associated with pancreatic cancer.

Despite these observations there still remains doubt as to the relevance of bile salt damage to the pancreatic duct; indeed Simpson (1983) and associates were hesitant to implicate the observed bile salt changes in the pathogenesis of acute pancreatitis. Several conclusions can however be made from the reports discussed.

1. Bile salts are toxic to the pancreatic duct mucosa and lead to increased permeability and mucosal ultrastructural changes.
2. Unconjugated bile salts are more toxic than the conjugated variety. Secondary bile salts are more damaging than primary salts.
3. Pressure increases bile salt damage.
4. Significant damage is produced by bile salts in the

concentrations found in bile and in the duodenum.

5. Bile salt induced duct damage may be important in the pathogenesis of acute gallstone pancreatitis and pancreatic cancer.

Materials and Methods

Object

To investigate the effects of bile salts, in particular glycodeoxycholic acid, on pancreatic duct integrity using the in vivo perfusion of the rat bile-pancreatic duct.

Experiment

The rat BPD was prepared as previously described in fig. 28. The pressure of infusion was kept low (i.e. <10 cm H_2O) in all these experiments with bile salts. Groups of five animals were used for each bile salt and concentration studied. The experimental perfusion was thus

period I - SPS perfusion x 1 hour

period II - Bile salt solution x 20 minutes

period III - SPS perfusion x 1 hour

Solutions

(1) glycodeoxycholate (Sigma G - 3258) was added to SPS to give concentrations of 5, 10, 20, 30 mM. Each solution was freshly prepared under clean conditions before use.

(2) taurocholate (Sigma T - 4009) was added to SPS to give a concentration of 10 mM.

(3) deoxycholate (Sigma D - 2510) was added to SPS to give a concentration of 10 mM.

Glycodeoxycholate was studied in detail as this bile salt may be the key detergent in producing membrane damage (see later). The concentrations of bile salts studied are those known to occur in human bile, where most

bile salts are bound to glycine (Carey 1973).

PMB damage

This was assessed as previously described. Damage to the PMB was indicated by

: increased anionic flux of Cl^- and HCO_3^-

: change in pD

: ultrastructural alterations.

Results (table 25, figs 35A-C)

Glycodeoxycholate perfusion produced a significant ($P<0.001$) increase in duct permeability compared with the control values. This increase was produced even by the relatively low 5 mM concentration. As the concentration of glycodeoxycholate was increased, so did the permeability of the pancreatic duct also increase. When a 20 mM solution of glycodeoxycholate was perfused the Cl^- flux was increased three-fold and the HCO_3^- flux two-fold. Moreover, after perfusion with 20 and 30 mM glycodeoxycholate the collected effluent in period III was discoloured. This indicated pronounced epithelial damage as in all control experiments the effluent remained colourless.

Electron microscopy revealed significant changes in the duct ultrastructure after glycodeoxycholate perfusion (fig. 36). At a concentration of 5 mM there were flattened epithelial cells and loss of microvilli and at a concentration of 10 mM the intercellular spaces were widened and the epithelial cells were swollen. After perfusion with a 20 mM solution epithelial disruption was noted and with the 30 mM solution cell shedding into lumen and an oedematous subjacent lamina propria were pronounced.

The degree of ultrastructural damage correlated with the increase in anionic flux and the change in pD. These results show that although the maximum damage to the duct occurred with glycodeoxycholate concentrations of 20 mM or above, there were still significant alterations produced by the 5 mM concentration.

Taurocholate (10 mM) perfusion increased duct permeability. This

increase was significantly ($P<0.02$) less than a corresponding 10 mM solution of glycodeoxycholate. Indeed 10 mM taurocholate produced changes that equated with 5 mM glycodeoxycholate in terms of ion flux and mucosal ultrastructure.

Deoxycholate (10 mM) produced changes in duct permeability and ultrastructure comparable to a 10 mM solution of the glycine conjugated salt. There was no apparent increased toxicity of the free bile acid over glycodeoxycholate at this concentration.

Results summary

1. Bile salts damaged the duct integrity. This damage was related to the concentration of the bile salts.
2. At equivalent concentrations, glycodeoxycholate was comparable to free deoxycholate and significantly more damaging than taurocholate.
3. A concentration of 20 mM glycodeoxycholate and above produced marked irreversible damage to the duct epithelium.
4. Damage to the PMB can be assessed by means of a change in ion flux and pD and by examination of mucosal ultrastructure. The quantitative changes in these indicators correlate well with each other. i.e. maximum flux ≡ maximum cell damage.

TABLE 25 Bile salts v. Anionic Flux (mean ± SD)

	mean change in net flux (μmol/cm/hr)		change in pD (mv)
	Δ J.Cl^-	Δ J.HCO_3^-	Δ pD
control (5)	+0.10±0.01	-0.07±0.01	-0.1±0.01
5mM GDC (5)	+1.20±0.08	-0.76±0.10	+0.38+0.02
10mM GDC (5)	+1.60±0.13*	-0.72±0.08	+0.70±0.02*
20mM GDC (5)	+2.09±0.32+	-1.31±0.11+	+0.80+0.05‡
30mM GDC (5)	+2.18+0.09	-1.49±0.11§	+0.78±0.12
10mM DOC (5)	+1.31±0.14Π	-0.64±0.07	+0.52±0.08Π
10mM TC (5)	+0.53+0.08¶	-0.27±0.04¶	+0.20+0.03¶

All bile salts v. control ($P<0.001$ for each)

GDC 10mM v. 5mM *$P<0.001$

GDC 20mM v. 10mM +$P<0.001$, ‡$P<0.02$

GDC 30mM v. 20mM §$P<0.02$

10mM DOC v. 10mM GDC Π$P<0.02$

10mM TC v. 10mM GDC ¶$P<0.01$

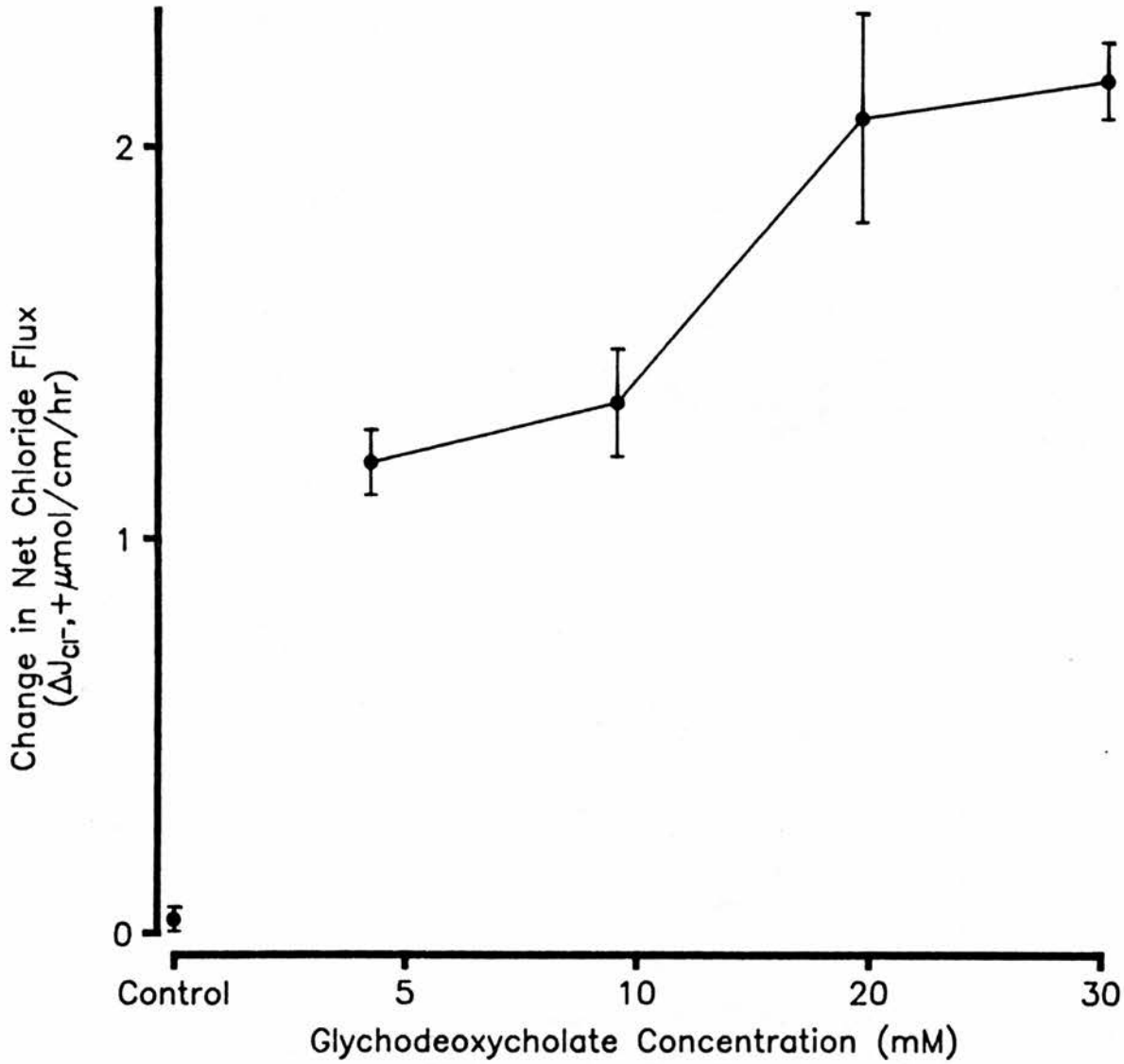


Fig. 35A GDC vs. chloride flux.

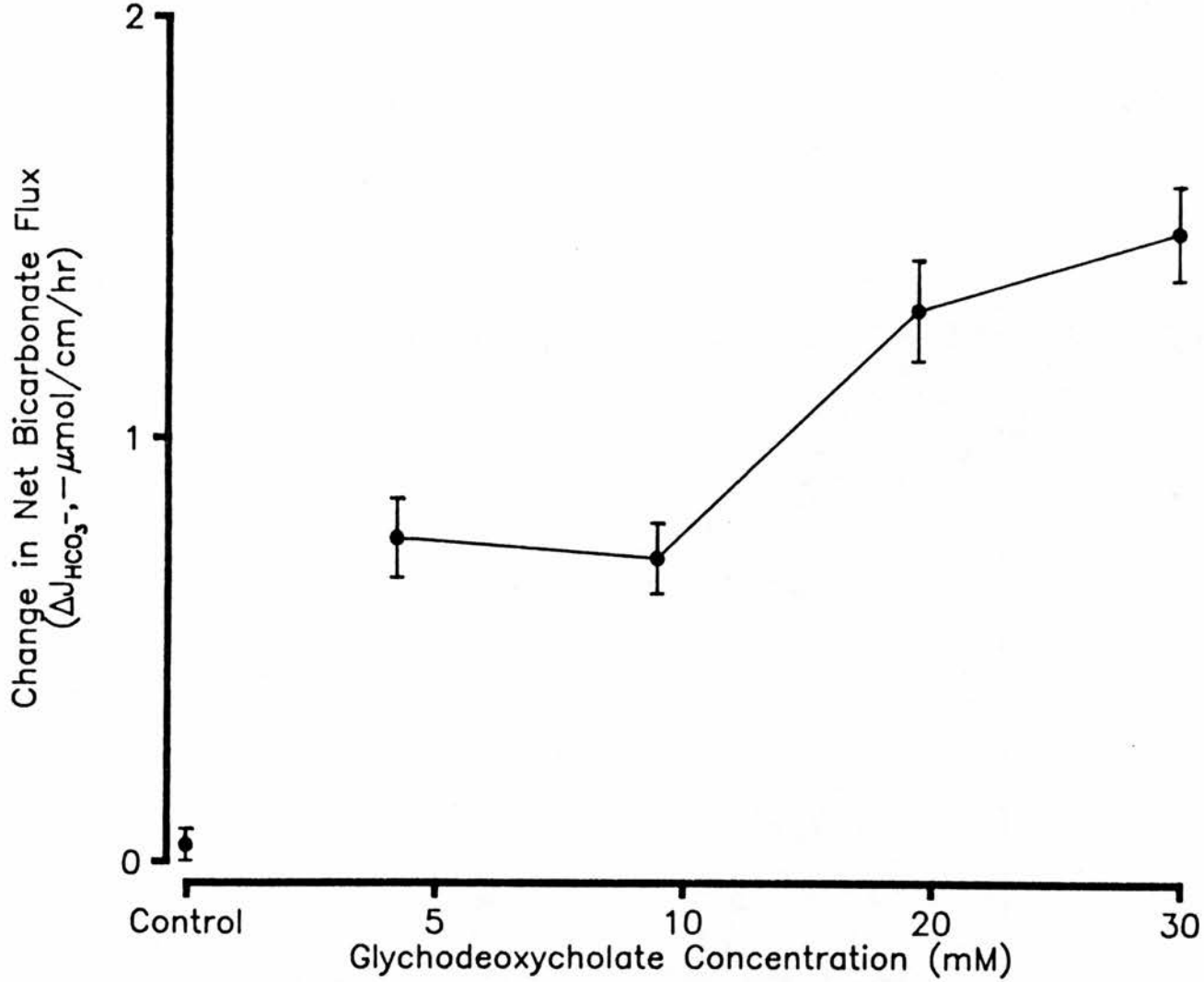


Fig. 35B GDC vs. Bicarbonate flux.

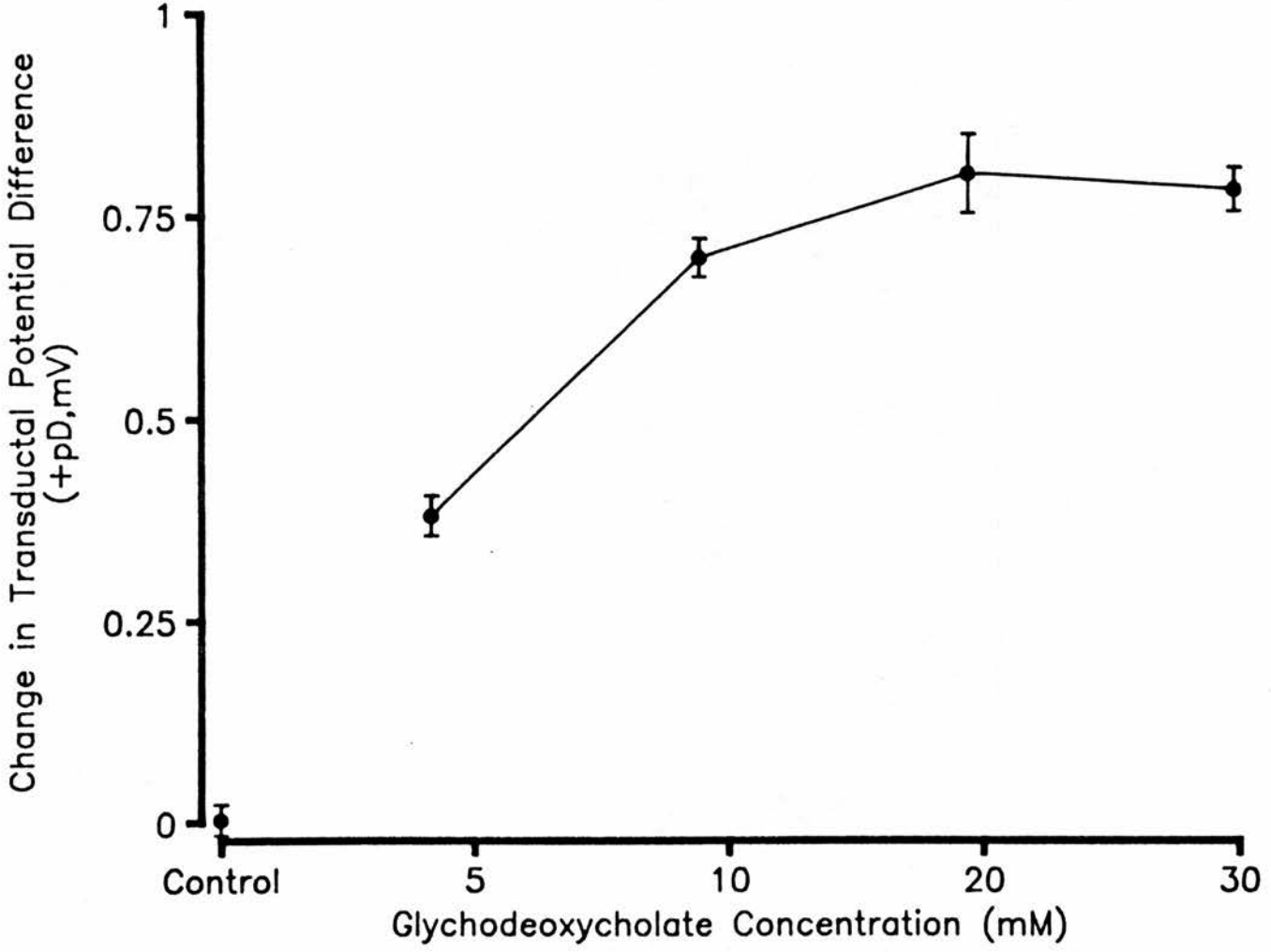


Fig. 35C GDC vs. potential difference.

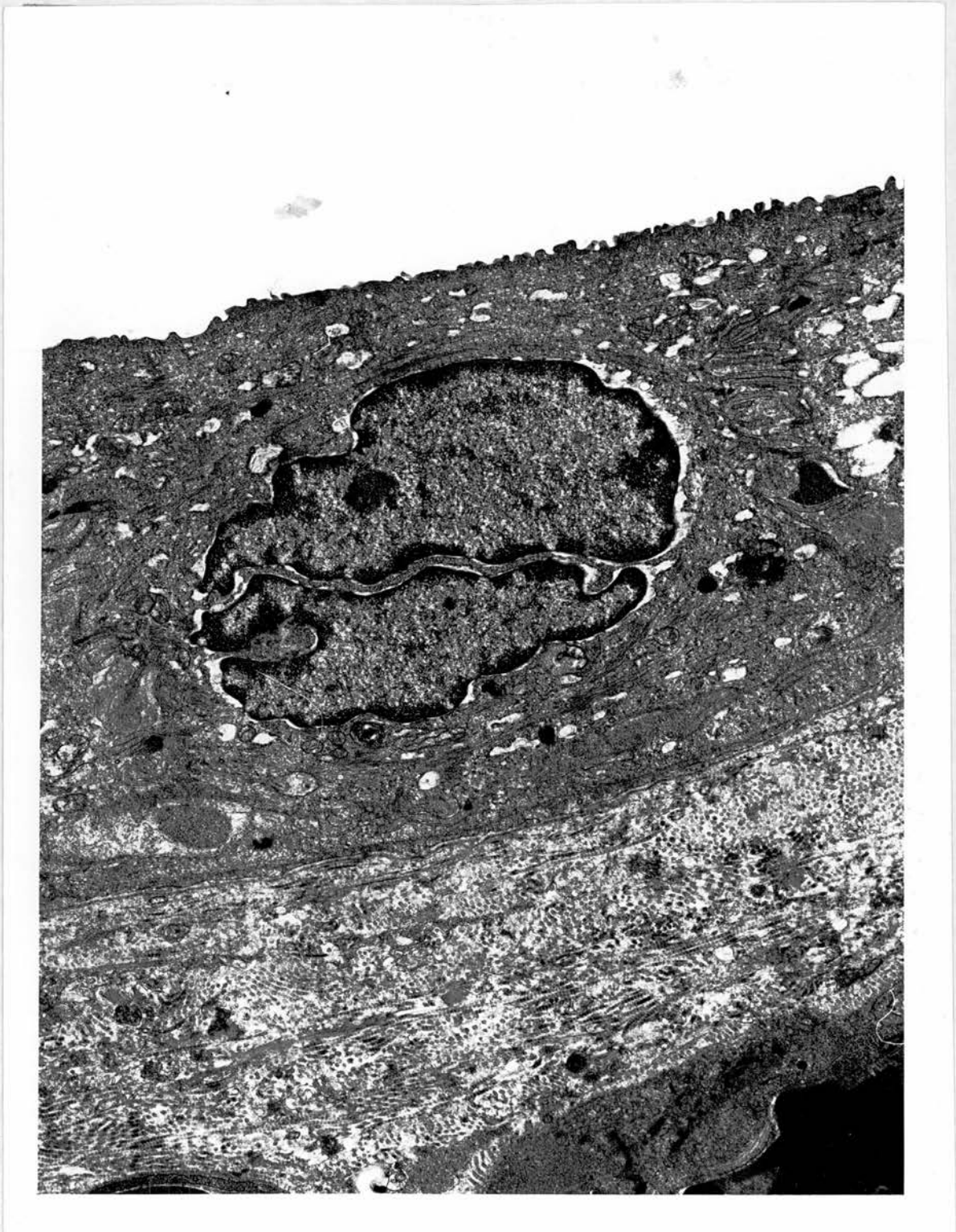


Fig. 36 Electron microscopy after perfusion of duct with bile salt (x 11250) (GDC)

Note epithelial flattening, loss of microvilli and oedema of cells.

Discussion

These experiments have demonstrated that bile salts are extremely toxic to the pancreatic duct. At equivalent concentrations glycodeoxycholate and free deoxycholate appeared to be equipotent and both were more toxic than taurocholate. A close study of glycodeoxycholate as opposed to other bile salts was made for several reasons -

(i) in man the glycine:taurine ratio is 3:1 (Carey 1973)

(ii) free bile acids rarely exist in bile (Hansson 1967)

(iii) secondary bile salts are more toxic than primary bile salts (Reber 1980)

(iv) patients with gallstones have an increase in the secondary bile salts (Heuman 1980).

It is possible that glycodeoxycholate might be the most important bile salt in producing pancreatic duct damage during the initiation of acute gallstone pancreatitis.

The degree of pancreatic duct damage seen on microscopy correlated closely with the increase in duct permeability. This observation is important when considering the validity of the experimental results. Although the degree of duct damage increased with higher concentrations of glycodeoxycholate these were all within presumptive pathophysiological concentrations. Even the low concentrations (5 and 10 mM) produced significant increases in anionic permeability. The ultrastructural changes observed at these concentrations were similar to those previously described (Simpson 1983, Reber 1981a). Glycodeoxycholate perfusion led to a consistent alteration of lateral intercellular relationships, a response analogous to active fluid transport (Simpson 1983, Tormey 1967). Even

when the intercellular spaces were widely distended, implying active transport across the epithelium, the cells remained in contact by tight junctions. Simpson (1983) felt that these changes were an adaptive response of a fully viable cell population. Whilst this is possible in the present experiment using the 5 mM concentration, the 10 mM solution also produced cellular swelling indicative of a degree of cell damage. Associated with these ultrastructural changes was a significant increase in duct permeability which is a factor in itself of import when considering duct extravasation. Simpson and co-workers (1983) have suggested that the pancreatic duct mucosa has many ultrastructural features in common with the biliary epithelium and it adapts to bile salt exposure by triggering physiological transport mechanisms. The results of perfusion with low concentrations of glycodeoxycholate support this theory, although we feel that the changes produced are important in themselves in the genesis of pancreatitis.

Duct perfusion with glycodeoxycholate at high concentrations of 20 and 30 mM produced much more severe damage, with associated ultrastructural alterations. Whilst it could be argued that low concentrations of glycodeoxycholate produced physiological adaptation in the duct cells, there is no doubt that these high concentrations are extremely toxic. After perfusion with the 20 and 30 mM solutions the collected effluent was discoloured. The implied severe epithelial disruption was confirmed by the electron microscopic appearances of cell shedding and debris in the lumen. Indeed in several ducts the subjacent basal lamina and lamina propria were damaged themselves.

The results of this series of experiments parallel recent experiments in

vitro (Helenius 1975, O'Leary 1984) where surfactants cause changes in membrane permeability at intermediate concentrations and complete lysis at higher levels. Indeed O'Leary (1984) has shown that long term exposure to bile salt at low concentration induces pancreatic duct cell hyperplasia, whereas high concentration produces cell lysis and pancreatitis.

In conclusion several observations have been made concerning the effect of bile salts on bile-pancreatic duct integrity.

1. Bile salts increase duct permeability with corresponding ultrastructural alterations.
2. Low concentrations of glycodeoxycholate (5, 10 mM) produce changes that might be construed as physiological adaption. In contrast high concentrations (20, 30 mM) produce severe epithelial disruption.
3. The increase in duct permeability may be important in the pathogenesis of acute gallstone pancreatitis.

CHAPTER XI

PHOSPHOLIPASE A_2, LYSOLECITHIN AND THE PANCREATIC DUCT MUCOSAL BARRIER

Phospholipase A_2 (PLA_2) is an enzyme secreted by the pancreas as an inactive zymogen. After activation by trypsin, PLA_2 in the presence of bile salts removes a fatty acid from lecithin to give lysolecithin. This reaction is very rapid and occurs normally in the duodenum with 100 per cent of the bile lecithins being converted to lysolecithins (Poncelet 1972, Schmidt 1969); it could also occur in the biliary tree especially in the presence of infection. Lysolecithins as a group are extremely toxic to cell membranes (Reeman 1967) and it has been postulated that PLA_2 is of major import in the initiation of acute gallstone pancreatitis (Nevalainen 1980). The substrates of PLA_2, lecithin and cephalin, are the main lipid constituents of cell membranes and lecithin is an essential component of bile. Bile acids are activators of PLA_2. Thus bile or duodenal reflux could activate PLA_2 within the pancreas, and lead to toxic sequelae.

Duodenogastric reflux has been implicated in the pathogenesis of peptic ulceration. Various biliary constituents, including PLA_2, lysolecithin and bile salts, damage the gastric mucosal barrier (Boyle 1984, Silen 1981). Boyle and colleagues (1984) have measured the gastric levels of lysolecithin and PLA_2 in patients with gastric ulceration. The levels of these compounds were significantly higher than in normal controls suggesting that both PLA_2 and lysolecithin have a role in producing mucosal damage. Several authors have shown that lysolecithin can produce gastric mucosal injury (Boyle 1984, Davenport 1970a, Kivilaakso 1978) of greater severity

than that of bile acids alone. These observations on membrane toxicity in the stomach may be analogous to the situation in the pancreatic duct following bile or duodenal reflux.

Helenius and Simon (1975) have comprehensively evaluated the properties of detergents on biological membranes. Lysolecithin is a powerful detergent or surfactant with different membrane solubilization properties than bile salts. It appears to bind to membranes at very low concentrations and subsequently induce changes in permeability. At higher concentrations lysolecithin has more drastic effects such as membrane lysis and fusion. This lytic process appears to occur in 5 stages.

(i) surfactant monomer adsorbs to membrane.

(ii) surfactant momomer penetrates membrane.

(iii) induces change in molecular organization.

(iv) alters permeability.

(v) allows leakage and passage of molecules through membrane.

The effects of lysolecithin or PLA_2 on pancreatic duct integrity are unknown.

Poncelet and Thompson (1972) assessed the actions of bile phospholipids on the pancreas but not on the ducts. They demonstrated that the minimal concentration of lysolecithin necessary to produce pancreatic necrosis was 6 mg/ml (0.6%); well within pathophysiological concentrations. Aho and Nevalainen (1982) investigated the ultrastructural appearances of pancreatic tissue following infusion of PLA_2 or lysolecithin (PLA_2 50u/200µl, lysolecithin 2.5%). PLA_2 produced extensive cell damage. The injured cells contained swollen mitochondria and vesiculated endo-

plasmic reticulum with the most severely injured cells being totally disintegrated. These changes were very rapid and occurred within several minutes. Lysolecithin produced an identical picture suggesting that PLA_2 acted via the production of lysolecithin. These researchers commented that these changes were similar to those induced by bile salt.

Thus;

(i) Phospholipase A_2 acts on lecithin to produce the powerful detergent or surfactant lysolecithin.

(ii) PLA_2 and lysolecithin damage cell membranes and the gastric mucosal barrier.

(iii) PLA_2 and lysolecithin are toxic to the pancreas.

(iv) Their effects on pancreatic duct integrity remain unclear.

Materials and Methods

Object

To assess the effect of phospholipase A_2 (PLA_2) and lysolecithin on duct integrity using the in vivo experimental preparation described.

Experiment

The rat BPD was prepared as described in fig. 28. Perfusion pressure was low ($<$10 cm H_2O) in all these experiments. Groups of five animals were studied. The experimental perfusion was thus.

period I - SPS x 1 hour.

period II - PLA_2 or lysolecithin x 20 mins.

period III - SPS x 1 hour.

Solutions

1. Phospholipase A_2 (Sigma P-9139) was mixed with SPS to produce a final concentration of 500 units/ml.
2. PLA_2 and glycodeoxycholate

   PLA_2 was mixed with SPS to give a solution of 500 units/ml. Glycodeoxycholate was added to a strength of 10 mM.
3. Lysolecithin (L .4129)

   L -α- lysophosphatidyl choline was added to SPS to give a 1% solution.
4. Lysolecithin and glycodeoxycholate were added to SPS solution to give a final concentration of 1% lysolecithin and 10 mM glycodeoxycholate.

PMB damage

Damage to the pancreatic duct mucosal barrier was represented by

: increased flux of Cl^- and HCO_3^- ions.

: change in transductal pD.

: mucosal ultrastructure abnormalities.

Results (table 26, figs. 37A-C)

PLA_2

PLA_2 alone (low activity) produced a significant ($P < 0.001$) increase in permeability to Cl^- and HCO_3^- ions and reduced the pD. On electron microscopy the appearances were those of epithelial swelling with apical vacuoles indentified (figs. 38A and B). Junctional complexes were normal and the intercellular spaces widened. The underlying basement membranes and subjacent lamina propria were normal.

PLA_2 and glycodeoxycholate

The combination of glycodeoxycholate and PLA_2 produced more severe duct damage than when either was perfused alone. The ultrastructural appearances were those of marked epithelial vacuolation and cell disruption (figs. 38C-D). Large areas of cell shedding with exposure of the basement lamina were identified. These appearances were analogous to those observed after infected bile was perfused through the duct.

Lysolecithin

Lysolecithin alone produced marked alterations in duct permeability, and these changes were accompanied by striking ultrastructural abnormalities. The duct cells were vacuolated as were the intercellular spaces and marked cellular oedema was identified with swelling of mitochondria. The basement lamina and lamina propria demonstrated a varying degree of oedema. The appearances were similar to those seen after perfusion with a PLA_2 and glycodeoxycholate solution.

Lysolecithin and glycodeoxycholate

When lysolecithin and glycodeoxycholate were combined the resultant damage to duct integrity was greater than that after perfusion with

either alone. The effects were not additive, presumably because each alone had significant toxicity. The ultrastructural changes were those of cell swelling and oedema with both vacuolation and oedema of the basement lamina and lamina propia. Occasional areas of cell disruption were identified. These changes were not as pronounced as when PLA_2 and glycodeoxycholate were perfused in combination.

Results summary

1. PLA_2 alone produces slight damage to the duct integrity. When bile salt is added there is marked disruption of the duct structure.
2. Lysolecithin alone is toxic to the duct epithelium. This toxicity is increased in the presence of bile salts.
3. Lysolecithin and PLA_2 produce similar ultrastructural appearances suggesting that their modes of action are comparable.

TABLE 26 PLA_2/Lysolecithin v. Anionic Flux (mean ± SD)

	mean change in net flux (μmol/cm/hr)		change in pD (mv)
	Δ J.Cl^-	Δ J.HCO_3^-	Δ pD
control (15)	+0.10±0.01	−0.07±0.01	−0.1±0.01
10mM GDC (5)	+1.60+0.13	−0.72+0.08	+0.70±0.02
PLA_2 (5)	+0.60±0.05*	−0.54±0.04*	+0.41±0.02*
PLA_2 + GDC (5)	+2.41±0.20†	−1.83±0.14†	+1.34±0.11†
lysolecithin (5)	+1.66+0.08‡	−1.06±0.12‡	+0.78±0.09‡
lysolecithin + GDC (5)	+1.89±0.09‖	−1.34±0.11‖	+0.91±0.04‖

PLA_2 v. control * $P<0.001$

PLA_2/GDC v. PLA_2 or GDC alone †$P<0.01$

lysolecithin v. control ‡$P<0.01$

lysolecithin/GDC v. lysolecithin ‖ $P<0.02$

lysolecithin/GDC v. GDC ‖ $P<0.02$

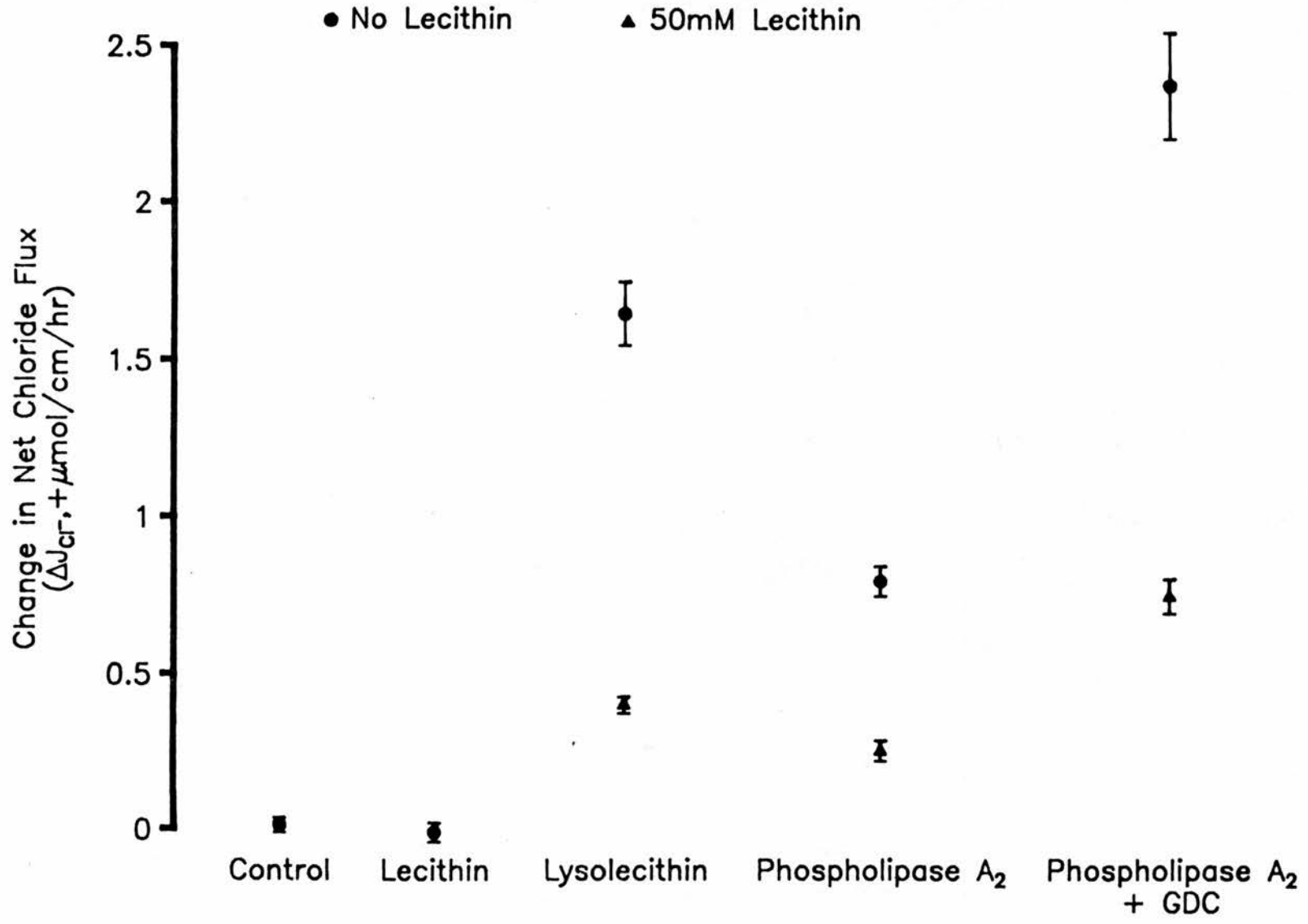


Fig. 37A PLA_2/Lysolecithin vs. chloride flux. (lecithin p 206)

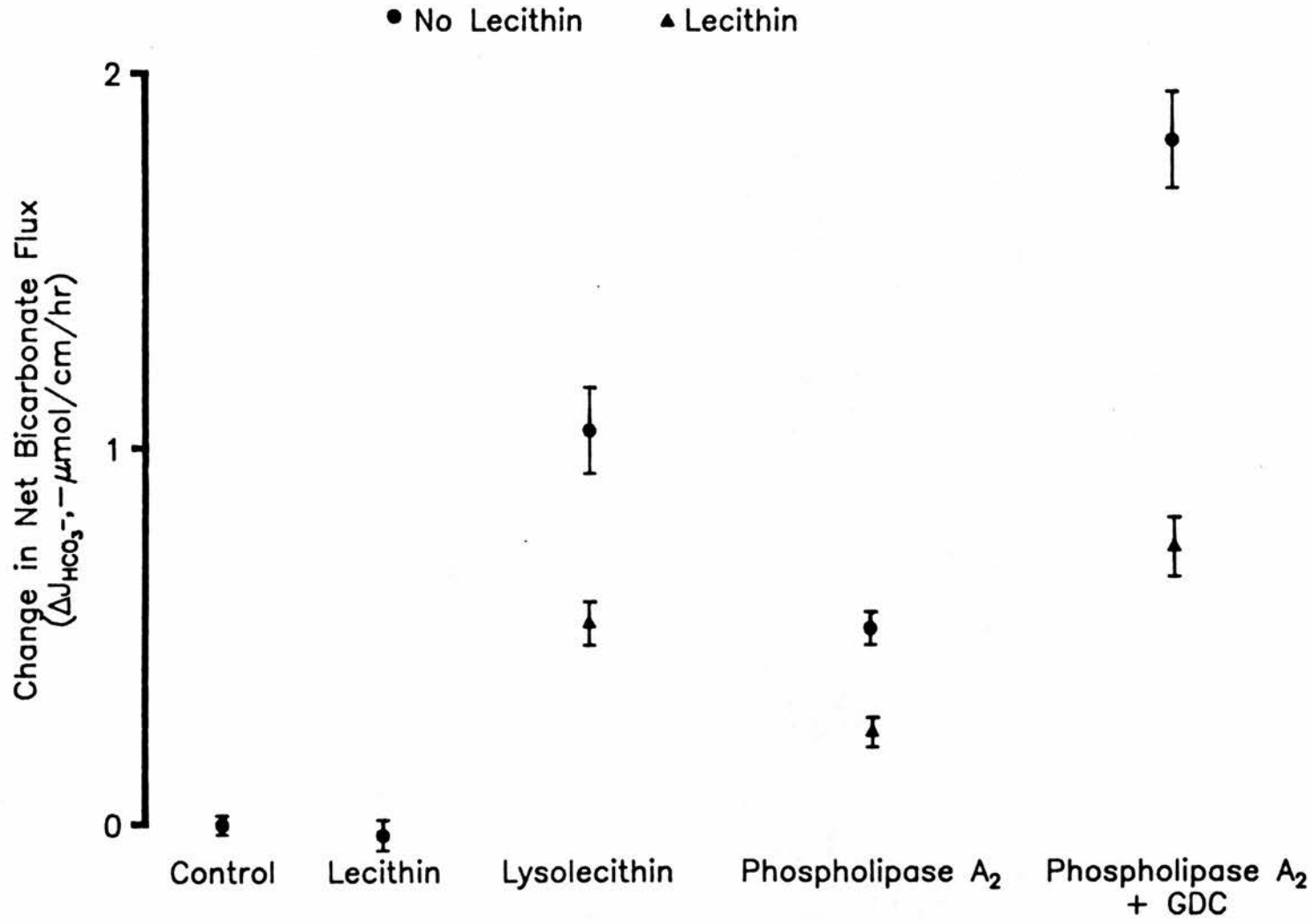


Fig. 37B PLA$_2$/Lysolecithin vs. Bicarbonate flux. (lecithin p206)

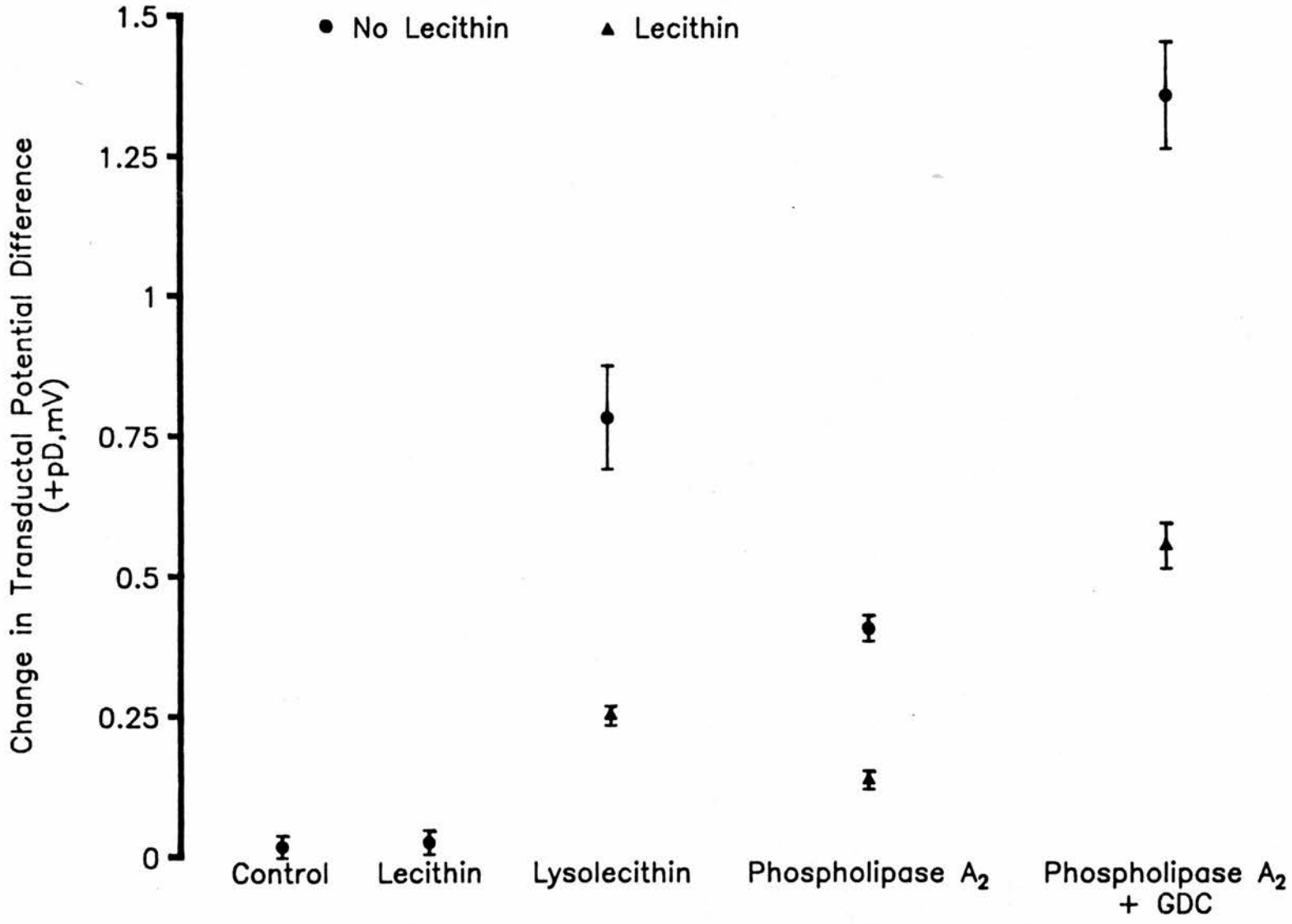


Fig. 37C PLA_2/Lysolecithin vs. potential difference. (lecithin p 206)

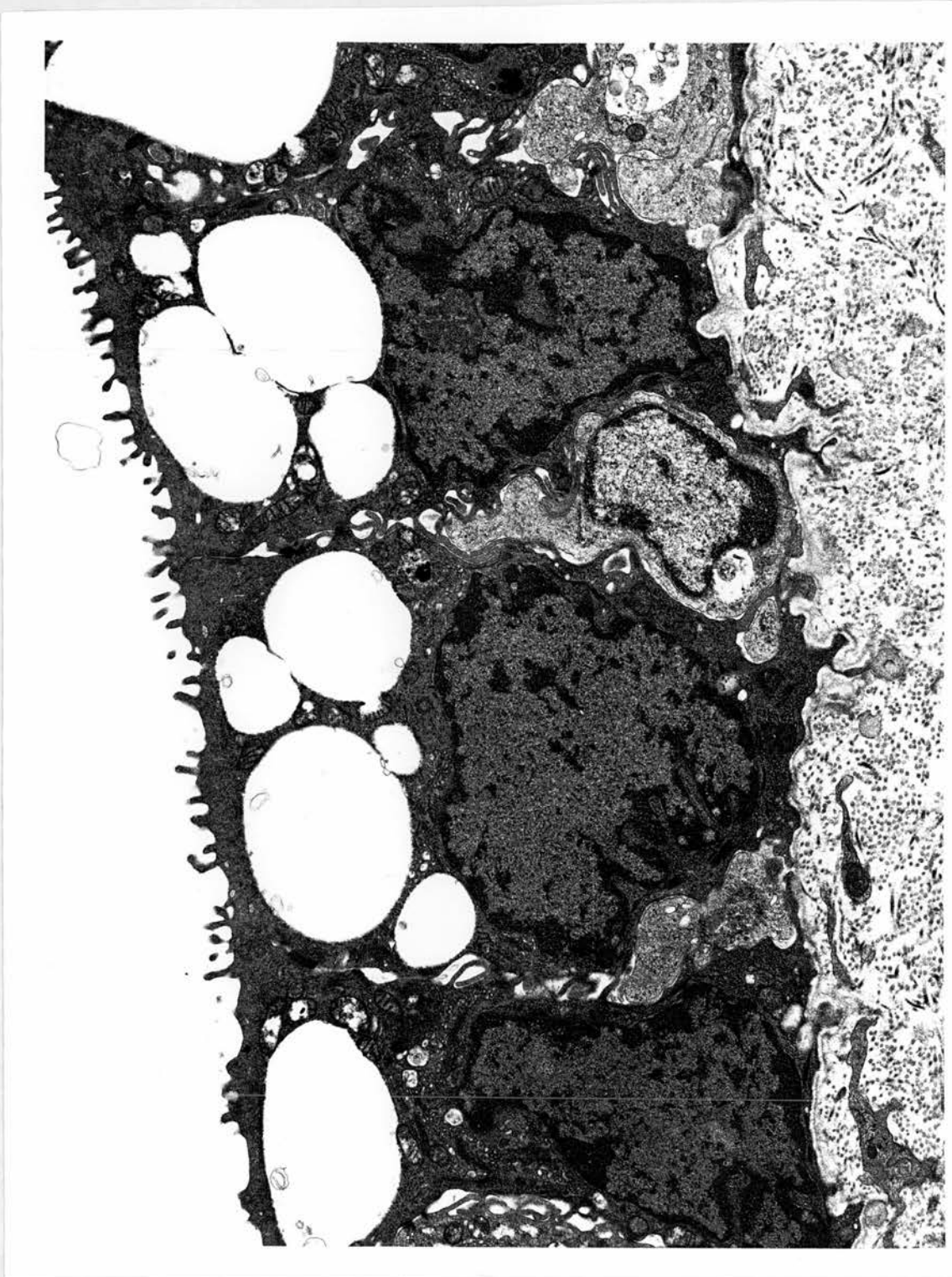


Fig. 38A Electron microscopy after perfusion of duct with lysolecithin (x 11250).

Note vacuolation of apical portions of cells.

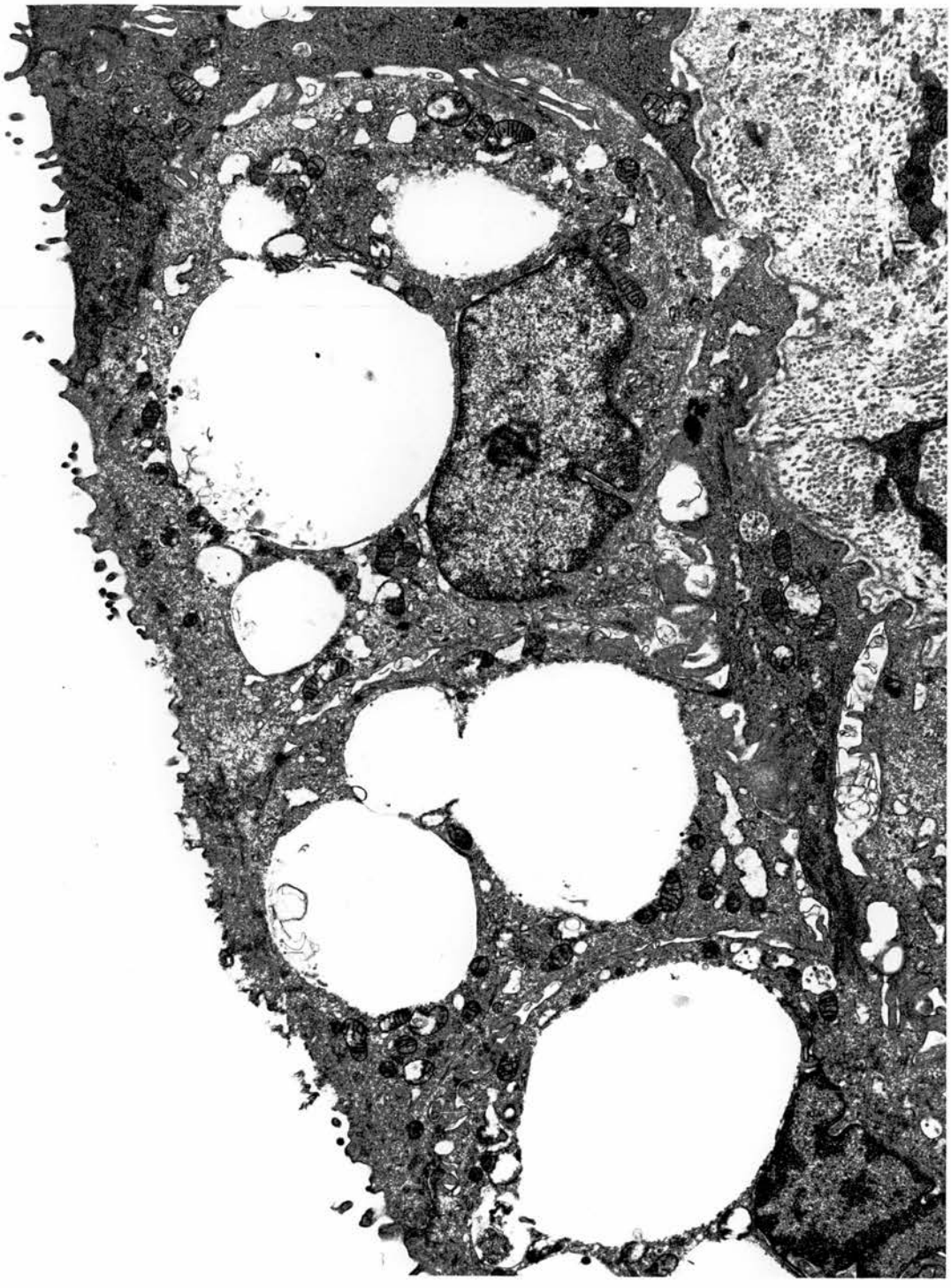


Fig. 38B Electron microscopy after perfusion of duct with lysolecithin (x 11250).

Note widespread vacuclation.

Fig. 38C Electron microscopy after perfusion of duct with Phospholipase A_2/GDC (x 4250).

Note separation and vacuolation of epithelium.

Fig. 38D Electron microscopy after perfusion of duct with PLA_2/GDC (x 11250).

Note cell loss and cell death.

Discussion

This study is the first evaluation of the toxic properties of PLA_2 and lysolecithin to the pancreatic ducts. As reflux of bile and/or duodenal juice into the pancreatic ducts appears to be the initiating step in gallstone pancreatitis, these observations may have significance in the pathogenesis of this disease.

Infusion of PLA_2 alone, at physiological concentrations (Aho 1982), produced little damage to the duct, almost certainly because of its inactivity in the absence of bile salts. In contrast when PLA_2 was infused in the presence of bile salt, and presumably fully activated, there was a marked impairment in duct integrity. The changes in ionic flux and transductal pD were greater than the sum of the individual effects. This suggest that both PLA_2 and bile salt act in concert to attack cell membranes and cause severe duct damage. The changes in ion flux were extremely marked and were some of the highest observed using this preparation. On electron microscopy there was widespread epithelial disruption with damage to the subjacent tissues. These ultrastructural appearances are analogous to those described by Aho and associates (1982).

Lysolecithin alone produced significant pancreatic duct damage at concentrations (1%) well within those seen in the duodenum and in the biliary tree (Poncelet 1972, Boyle 1984, Haverback 1960). There was a marked increase in pancreatic duct permeability as reflected by changes in anionic flux and transductal potential difference. This increase in permeability was of a similar magnitude to that observed after perfusion with a 10 mM solution of glycodeoxycholate. The ultrastructural changes

associated with barrier disruption were comparable to those of active PLA_2 in that duct cells were vacuolated with changes in the lateral cellular interdigitations being prominent. Cell damage was further evidenced by mitochondrial swelling. The underlying basement lamina and lamina propia were oedematous suggesting that they too were damaged by the effects of lysolecithin. Junctional complexes appeared normal suggesting that some degree of recovery was possible after removal of the detergent from the duct. These changes were increased when glycodeoxycholate was added although not to the same degree as seen after its addition to PLA_2, presumably resultant on differing active concentrations of PLA_2 and lysolecithin. Following glycodeoxycholate and lysolecithin perfusion occasional areas of cell disruption were identified. Bile salts and lysolecithin are the two most powerful natural detergents (Helenius 1975) and they appear to damage the duct cells of the pancreas in concentrations similar to those found in bile and duodenal juice.

Our previous observations have shown that sterile bile is less damaging than bile salt and lysolecithin to the pancreatic duct mucosal barrier. This may be due to the protective action of certain other biliary constituents, e.g. lecithin, which will be evaluated in the next chapter. It has been well described that a combination of pancreatic juice and bile is more injurious to gastric mucosa (Lawson 1964) and the pancreas (Elliott 1957) than bile alone. We believe that this is as a result of PLA_2 action on bile with the production of lysolecithin. Both lysolecithin and activated PLA_2 produced significant damage to the bile-pancreatic duct.

This study can be summarized as follows;

1. PLA_2 after activation by bile salts was extremely toxic to the pancreatic duct.
2. Lysolecithin produced duct damage which was of a similar magnitude to that following perfusion with a 10 mM glycodeoxycholate solution.
3. The changes induced by PLA_2 and lysolecithin were similar suggesting that PLA_2 acts via lysolecithin production. These changes are different to those of bile salts.
4. Lysolecithin and active PLA_2 may produce damage to the pancreatic ducts and so play a part in the initiation of acute gallstone pancreatitis.

CHAPTER XII

CYTOPROTECTION OF THE PANCREATIC DUCT EPITHELIUM

"Cytoprotection": The concept of "cytoprotection" was introduced by Robert (1976) in an attempt to explain how the gastric mucosa could be protected when the gastric mucosal barrier was either broken or the mucosa was exposed to potentially harmful circumstances. A variety of factors appear to be important in maintaining the cellular integrity of gastric epithelium including mucus, mucosal blood flow, the cells themselves, tight junctions, prostaglandins and antihistamines (Moody 1981).

Prostaglandins: Prostaglandins, a family of long chain unsaturated fatty acids derived from arachidonic acid, have been known to affect gastrointestinal function since their original detection in 1934. Prostaglandin E_2 (PGE_2) and its derivatives have been shown to have potent antiulcer activity; a protective action distinct from its anti-secretory actions and termed "cytoprotection" (Robert 1976, Miller 1979). Prostaglandins have the ability to protect the cells of the gastrointestinal epithelium against a variety of potentially noxious agents which otherwise have the capability of producing cellular damage and necrosis e.g. bile salts, aspirin, hypertonic solutions and ethanol (Moody 1981). Recently Lacy and Ito (1982) examined the ultrastructural cytoprotection of PGE_2 derivatives on ethanol-induced gastric damage. They found that PGE_2 failed to alter surface epithelial damage but did reduce deep damage. This finding is interesting in that there was no general cytological protection of gastric epithelium. Further evidence on the importance of prostaglandins in maintaining gastric mucosal integrity has been afforded by the finding

of reduced "cytoprotection" when a potent anti-prostaglandin such as indomethacin is present (Miller 1984). Indeed, endogenously produced prostaglandins appear vital in the maintenance of the gastric mucosal barrier. These observations suggest that prostaglandins serve a physiological role in maintaining the normal mucosal integrity of the digestive tract rather than inducing cytoprotection solely as a pharmacological effect (Robert 1979). Despite much investigation the actual mechanism for prostaglandin - induced cytoprotection is unknown although it has been suggested that prostaglandins may act via a change in mucosal blood flow, a reduction in epithelial permeability, an inhibition of active ion transport or an alteration in mucus secretion (Miller 1979). Oral administration of a PGE_2 analogue stimulates gastric mucus secretion (Allen 1980). Gastric mucus is a visco-elastic polymeric gel secreted from surface epithelial cells and appears to have a distinct role in cytoprotection. Thus the cytoprotective action of PGE_2 might be as a result of its effect on mucus secretion.

Although most of the research on intestinal prostaglandins has concentrated on the stomach, interest has grown regarding prostaglandin activity in the pancreas and biliary tract (Uddin 1983a). Prostaglandins (PGE_2) have several important actions on the pancreas including vaso-dilatation and increased blood flow, inhibition of secretin-induced secretion, varying effects on enzyme secretion, relaxation of the circular muscles of the biliary and pancreatic duct, and a possible increased mucus secretion.

There has been much recent interest concerning the role of prostaglandins in acute experimental pancreatitis. Standfield and Kakkar (1983) demonstrated that PGE_2 reduced the severity of ethionine-induced

pancreatitis, and postulated that membrane stabilisation induced by prostaglandins was an important factor. They further illustrated that the administration of parenteral PGE_2 to humans was associated with a significant reduction in cell membrane enzyme levels of N-acetyl β glucosaminidase (a marker of lysosomal stability), malate dehydrogenase (a marker of plasma membrane stability). The beneficial effect of protaglandins in experimental pancreatitis was further evidenced by Coelle (1983), Manabe (1980), Brems (1983), Robert (1983) and Olazabal (1983). Furthermore, indomethacin increases the severity of experimental pancreatitis (Olazabal 1980, Coelle 1983). Whilst these results indicate that the prostaglandin system is of importance in the outcome of pancreatitis, further studies are needed to evaluate the role of prostaglandins in the pathophysiology of pancreatitis.

The pancreatic duct mucosal barrier (PMB) is damaged by contact with bile salts, aspirin, ethanol, lysolecithin and phospholipase A_2 (Reber 1980). Researchers from Reber's laboratory in Columbia, Missouri have recently investigated the effects of prostaglandins on the PMB (Mosley 1981, Tweedie 1981, Reber 1981a). Tweedie (1981) showed that the administration of an intravenous PGE_2 analogue prevented the increased permeability in the pancreatic duct produced by aspirin. The effect was dose-related and at the highest dose used (50 μg/kg/hour) was essentially complete. Reber (1981a) reported that 16, 16 DM PGE_2 (a synthetic analogue of PGE_2) reduced the ultrastructural abnormalities induced by glycodeoxycholate and, in particular, the intercellular spaces were normal. Thus a morphological correlation between the ultrastructural improvement and the change in duct permeability induced by prostaglandins was demonstrated. Mosley (1981) evaluated the effect of 16, 16 DM PGE_2 on

damage to the PMB. Intravenous prostaglandin (50 μg/kg/hour) partially protected the mucosal barrier against damage produced by aspirin and bile salt. He also demonstrated that cimetidine was partially cytoprotective to the PMB.

In contrast to these reports on the efficacy of pancreatic duct cytoprotection afforded by PGE_2, were the recent observations of Olazabal (1983). He studied the effects of PGE_2, PGI_2 and indomethacin on deoxycholic acid-induced damage to the rat bile-pancreatic duct using a preparation similar to that in the present experiment (although less refined in the detection of damage to the mucosal integrity). Prostaglandin E_2 or I_2 was given by three routes (intravenous, intra-arterial and intraductal) before bile salt perfusion. Indomethacin was administered intravenously before perfusion. Neither prostaglandin nor indomethacin affected the alterations in duct integrity induced by deoxycholic acid. There are several possible reasons why his results were contrary to those of Reber and colleagues; (i) the dose of prostaglandins used was smaller, (ii) PGE_2 was used instead of the more potent analogue 16, 16 DM PGE_2, (iii) the assessment of mucosal integrity was relatively crude, (iv) there may be species differences in the response to prostaglandins (rat vs cat).

These studies suggest that prostaglandins (in particular PGE_2 and its analogues) are partially protective to the pancreatic duct epithelium. However further studies, using refined methods for assessing duct integrity, are needed to fully assess the physiological role of prostaglandins in pancreatic duct cytoprotection.

Lecithins (phosphatidylcholine): are an integral constituent of human bile and phospholipids, produced by hepatocytes, exist as 96% lecithin (phosphatidylcholine), 3% lysolecithin and 1% phosphatidyl ethanolamine. Lecithin secretion into bile is dependent on bile salts, although some independent secretion occurs (Smith 1981). The major role of phospholipids in bile is that of solubilization of cholesterol in mixed micelles. However, it has been recently suggested that biliary phospholipids (henceforth known as lecithin) are also important in reducing the toxicity of bile salts to epithelial membranes and may be involved in maintaining the integrity of the gastro-intestinal mucosa (Martin 1981).

Martin and Marriott (1981) studied the effects of lecithin (phosphatidyl-choline) on the direct toxicity of bile salt and lysolecithin to biological membranes. Lecithin reduced the toxicity of the bile salt, possible as a result of mixed micelle formation (Helenius 1975). The mixed micellar aggregates reduce the rate at which the intact membrane is damaged because of the reduced solubilizing capacity of bile salt micelles for membrane components. One mole of bile salt can solubilize up to a maximum of 2 moles of lecithin (Martin 1981) e.g. 50 mM lecithin should be completely solubilized by 25 mM glycodeoxycholate. In contrast 1 molecule of lecithin can solubilize up to 10 molecules of lysolectihin (Helenius 1975). Lecithin also reduced the toxcity of lysolecithin to membranes by again decreasing the solubilizing capacity for membrane protein and lipid components. Martin and Marriott concluded their report by postulating that lecithin in bile might be of importance in maintaining intestinal integrity in vivo. These results may be significant with possible bile or duodenal reflux into the pancreatic duct system.

More recently Marriott and co-workers (1984) have reported on the protective action of lecithin on the gastric mucosa. The addition of 20 mM lecithin to 20 mM taurodeoxycholate significantly stabilized the gastric mucosal barrier by reducing the effects of taurodeoxycholate on potential difference and ionic flux. These experiments have important implications in mucosal protection as to date there has been little investigation of the protective properties of the lecithin molecule.

Heuman and associates (1980) studied the permeability of the rabbit gallbladder after treatment with bile salt. Bile salt markedly increased the permeability to dextran molecules of molecular weight 3000. When lecithin (50 mM) was added to the bile salt there was a significant reduction in gallbladder permeability to dextran. They also demonstrated that patients with cholesterol gallstones had lower levels of lecithin in their bile than normal controls (35 mmol/l vs 53 mmol/l). These results confirmed earlier reports by Ammon (1979) that lecithin protected the gallbladder mucosa against the potentially damaging effects of bile salts. Thus the lecithin to bile salt ratio might be critical in determining bile toxicity to membranes. For example, the addition of lecithin to bile will reduce the bile salt to lecithin ratio and the amount of bile salts in monomer and simple micellar form. This will result in a reduction of the overall detergent effect on the biliary epithelium. As gallbladder epithelium has many features in common with that of the pancreatic duct the implications of this study in the maintenance of pancreatic duct integrity are far reaching. Recently Sjödahl and Tagesson (1983) have reviewed factors responsible for the development of acute cholecystitis. They found that lecithin was crucial in counteracting the detergent properties of lysolecithin and bile acids. If the

concentration of lecithin was decreased, the overall detergent capacity of the gallbladder bile was considerably increased. It remains unproven whether the same sequence of events occurs in the bile of patients with gallstone pancreatitis.

Poncelet and Thompson (1972) have shown that lecithin will reduce the toxicity of lysolecithin to the pancreas. Lecithin completely suppressed the toxicity of lysolecithin and prevented the development of acute pancreatitis. Since lysolecithins are produced by the action of phospholipase A_2 on lecithin a high concentration of lysolecithins indicates a low value of lecithins. Thus lecithins may also be important in modifying lysolecithin induced damage to the pancreas. These observations suggested that further evaluation of the effects of lecithin on the pancreatic ducts was needed. In particular the relationship of lecithin to bile salts and lysolecithin required clarification.

In summary there are several points on mucosal protection in the pancreatic duct which should be emphasized.

1. Prostaglandins of the E_2 series are definitely cytoprotective to the gastric epithelium.
2. The mechanism of prostaglandin induced cytoprotection is unknown.
3. PGE_2 and its analogues may also protect the pancreatic duct.
4. Lecithins protect gastric mucosa from bile salt and lysolecithin damage.
5. Lecithins reduce the bile salt-induced increase in gallbladder permeability.
6. The lecithin:lysolecithin and lecithin:bile salt ratios may be important in determining bile toxicity.

7. The effects of PGE_2 and lecithin on the pancreatic duct are not clear, and further study is needed to evaluate their importance in the pathogenesis of acute gallstone pancreatitis.

Materials and Methods

Object:

To evaluate the effects of prostaglandin E_2 and lecithin (phosphatidylcholine) on damage to the pancreatic duct epithelium induced by

(i) bile salts and

(ii) PLA_2 and lysolecithin.

Experiment:

The experimental preparation described in fig. 28 was used in all experiments with a low pressure infusion (<10 cm H_2O); N = 5 in each group.

The experimental periods were therefore;

period I - SPS x 1 hour.

period II - Bile salt or lysolecithin/PLA_2) + lecithin or PGE_2

period III - SPS x 1 hour.

Solutions used:

1. glycodeoxycholate at concentrations of 5, 10, 20, 30 mM.
2. lysolecithin - 1% solution.
3. PLA_2 - 500 units/ml alone and in combination with 10 mM glycodeoxycholate.
4. Lecithin (L -α- phosphatidylcholine, Sigma P - 4139) was mixed with SPS to produce a 50 mM solution. This was added to

   either (a) glycodeoxycholate (5-30 mM),

   or (b) PLA_2 solution,

   or (c) lysolecithin solution.

   and infused into the bile-pancreatic duct in period II. A control experiment using lecithin alone was used to compare results.

and infused into the bile-pancreatic duct in period II. A control experiment using lecithin alone was used to compare results.

5. Prostaglandin E_2 (PGE_2) (Sigma, P - 5640) was made up to a 0.01 M stock solution with absolute ethanol and stored at -20°C. This was diluted with SPS to the final concentration required immediately before use. The dosage of PGE_2 used was 100 µg/kg/hr. (the concentration of ethanol in the infused PGE_2 was approximately 3.8%). The prostaglandin was infused intraductally for 20 minutes following period I and then the substance under test (period II). The ducts were thus pre-treated with PGE_2.

Barrier damage was assessed by

: change in anionic flux of Cl^- and HCO_3^-

: transductal potential difference (pD)

: mucosal ultrastructure

Results

Lecithin and glycodeoxycholate (table 27, figs 39A-C)

When lecithin alone was perfused through the bile-pancreatic duct there was no change in duct permeability and the electron microscopic appearance was normal. The addition of lecithin to varying concentrations of bile salts significantly reduced the changes in anionic flux of chloride ($P < 0.001$), bicarbonate ($P < 0.001$) and the transductal potential difference ($P < 0.001$) induced by bile salt alone. This protection was significant with each concentration of glycodeoxycholate and was most noticeable with high glycodeoxycholate concentrations where the changes in ion flux and pD were greatest. Ultrastructural examination confirmed the protective qualities of lecithin. The cellular changes produced by low glycodeoxycholate concentrations were much reduced with less oedema and flattening of the duct epithelium. However, the most spectacular changes were noted with high glycodeoxycholate concentrations (20, 30 mM). At these concentrations lecithin prevented the epithelial disruption, cell shedding and oedema of the subjacent lamina propria. The electron microscopic appearance was similar at each glycodeoxycholate concentration when lecithin was used as a mucosal protector. (figs 40A-C). Thus lecithin had a marked protective action on bile salt induced duct damage.

PGE_2 and glycodeoxycholate (table 28, figs 41A-C)

Prostaglandin E_2 alone had little effect on duct integrity as assessed by anionic flux, pD and mucosal ultrastructure. PGE_2 significantly ($P < 0.01$) protected against 5 mM glycodeoxycholate induced damage. At other concentrations of glycodeoxycholate, PGE_2

appeared to be partially protective. For example with 10 mM glycodeoxycholate there was a significant reduction in Cl^- flux ($P < 0.01$) and a change in pD ($P < 0.01$) whereas HCO_3^- flux was unaltered. The same inconsistency in mucosal protection was noted on electron microscopy. PGE_2 markedly reduced the ultrastructural damage produced by 5 mM glycodeoxycholate. In contrast, the protection offered for 10 mM glycodeoxycholate was less pronounced. With 20 mM glycodeoxycholate, PGE_2 precented epithelial disruption with the only changes observed being those of cell swelling and widening of the intercellular spaces. The effect of PGE_2 on 30 mM glycodeoxycholate induced damage was much less obvious as the epithelium was still disrupted with exposure of the basement lamina.

Thus prostaglandin E_2 (given intraductally) had an inconsistent protective action on glycodeoxycholate induced damage. A degree of mucosal protection was afforded with glycodeoxycholate concentrations below 20 mM. The protective action of PGE_2 was significantly less pronounced than that of lecithin at all concentrations of glycodeoxycholate, as demonstrated in the figures for reduction in mucosal permeability:

Glycodeoxycholate concentrations (mM)	ΔJCl^- L	PG	$\Delta JHCO_3^-$ L	PG	ΔpD L	PG
5	48%	45%	42%	46%	63%	26%
10	64%	28%	20%	8%	75%	57%
20	65%	31%	54%	18%	45%	10%
30	62%	23%	63%	8%	46%	0%

Figures shown represent the percentage reduction in permeability or change in pD. High values represent a high degree of "cytoprotection".

(L = lecithin, PG = prostaglandin E_2)

PLA_2/lysolecithin and lecithin (Table 29, fig. 37A - C). Lecithin (50 mM) significantly reduced the damage to duct integrity produced by PLA_2 (alone and in combination with glycodeoxycholate) and lysolecithin. All indices of barrier damage were significantly reduced ($P<0.001$ for each). There were reductions in mucosal permeability of:

	$\Delta J.\ Cl^-$	$\Delta J.\ HCO_3^-$	ΔpD
PLA_2	57%	56%	66%
PLA_2 + glycodeoxycholate	69%	62%	59%
Lysolecithin	75%	49%	68%

(Figures shown represent the percentage reduction in permeability or change in pD).

The changes in mucosal permeability produced by the addition of lecithin are comparable for these different agents suggesting a

similar mode of action.

The protective action of lecithin was further emphasized by ultrastructural examination of the duct cells. Lecithin prevented the epithelial swelling and vacuolation induced by PLA_2, and the epithelial disruption produced by the PLA_2/glycodeoxycholate solution was markedly reduced as was that of the lysolecithin. Thus lecithin had a marked protective action against phospholipase A_2 and lysolecithin-induced damage to the mucosal barrier.

Results summary

1. Lecithin (50 mM) significantly protected the pancreatic duct against all detergent-induced damage.
2. Prostaglandin E_2 given intraductally had a variable protective action against glycodeoxycholate-induced damage to the mcuosal barrier. This protection was not apparent with 30 mM glycodeoxycholate and was always less than that afforded by lecithin.

TABLE 27 Lecithin vs. GDC damage (mean ± SD)

	mean change in net ion flux (μmol/cm/hr)				change in pD (mv)	
	Δ J.Cl^-		Δ J.HCO_3^-		Δ pD	
		LECITHIN		LECITHIN		LECITHIN
Control (15)	+0.10±0.01		-0.07±0.01		-0.1±0.01	
Lecithin (5)		-0.01±0.01		-0.03±0.01		+0.03±0.01
5mM GDC (5)	+1.20±0.08	+0.62±0.08*	-0.76±0.10	-0.44±0.03*	+0.38±0.02	+0.14±0.02*
10mM GDC (5)	+1.60±0.13	+0.58±0.06*	-0.72±0.08	-0.58+0.10+	+0.70±0.02	+0.17±0.03*
20mM GDC (5)	+2.09±0.32	+0.73±0.10*	-1.31±0.11	-0.60±0.10*	+0.80±0.05	+0.44±0.03*
30mM GDC (5)	+2.18±0.09	+0.84±0.13*	-1.49±0.11	-0.56±0.07*	+0.78±0.12	+0.42±0.03*

* $P<0.001$
\+ $P<0.02$
} Lecithin vs. No Lecithin

TABLE 28 PGE_2 vs. GDC damage (mean + SD)

	mean change in net ion flux (μmol/cm/hr)				change in pD (mv)	
	Δ J.Cl^-		Δ J.HCO_3^-		Δ pD	
		PGE_2		PGE_2		PGE_2
Control (15)	+0.10±0.01		−0.07±0.01		−0.1±0.01	
PGE_2 (5)		+0.04±0.01		+0.06±0.02		+0.1±0.02
5mM GDC (5)	+1.20±0.08	+0.66±0.05*	−0.76±0.10	−0.41±0.03*	+0.38±0.02	+0.28±0.10+
10mM GDC (5)	+1.60±0.13	+1.15±0.11*	−0.72±0.08	−0.66±0.10	+0.70±0.02	+0.30±0.09*
20mM GDC (5)	+2.09±0.32	+1.45±0.21*	−1.31±0.11	−1.08±0.09*	+0.80±0.05	+0.72±0.09+
30mM GDC (5)	+2.18±0.09	+1.69±0.23*	−1.49±0.11	−1.37±0.13	+0.78±0.12	+0.79±0.08

* $P<0.01$
\+ $P<0.05$
} PGE_2 vs. No PGE_2

TABLE 29 Lecithin vs. GDC/PLA_2/Lysolecithin damage (mean ± SD)

	mean change in net ion flux (μmol/cm/hr)				change in pD (mv)	
	Δ J.Cl^-		Δ J.HCO_3^-		Δ pD	
		LECITHIN		LECITHIN		LECITHIN
Control (15)	+0.10±0.01		−0.07±0.01		−0.1±0.01	
Lecithin (5)		−0.01±0.01		−0.03±0.01		+0.03±0.01
PLA_2	+0.60±0.05	+0.26±0.02*	−0.54±0.04	−0.24±0.03*	+0.41±0.02	+0.14±0.01*
PLA_2 + GDC	+2.41±0.20	+0.75±0.05*	−1.83±0.14	−0.74±0.08*	+1.34±0.11	+0.55±0.04*
Lysolecithin	+1.66±0.08	+0.41±0.03*	−1.06±0.12	−0.54±0.05*	+0.78±0.09	+0.25±0.02*

* $P<0.001$ Lecithin vs. No Lecithin

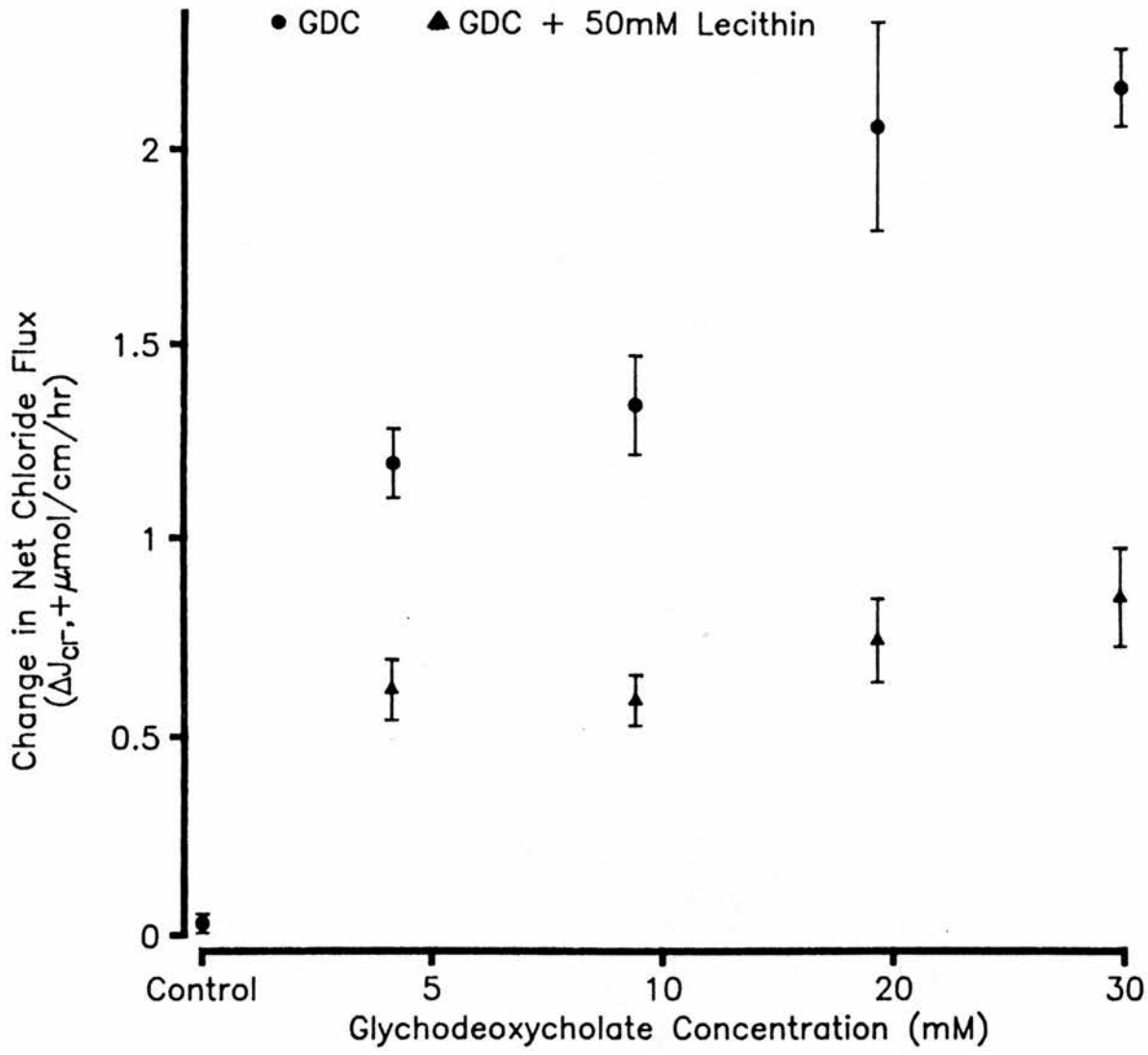


Fig. 39A GDC and lecithin vs. chloride flux.

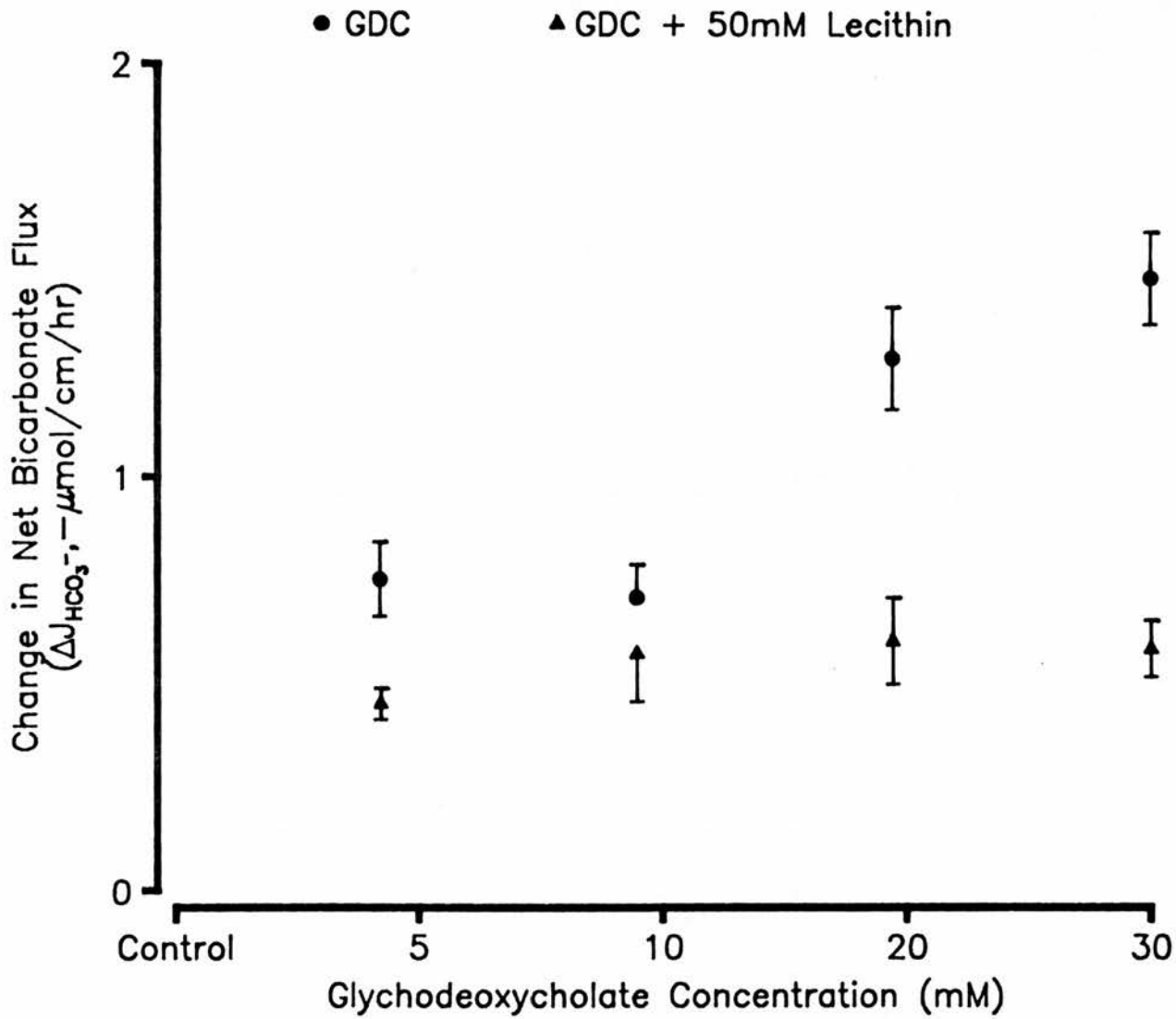


Fig. 39B GDC and lecithin vs. Bicarbonate flux.

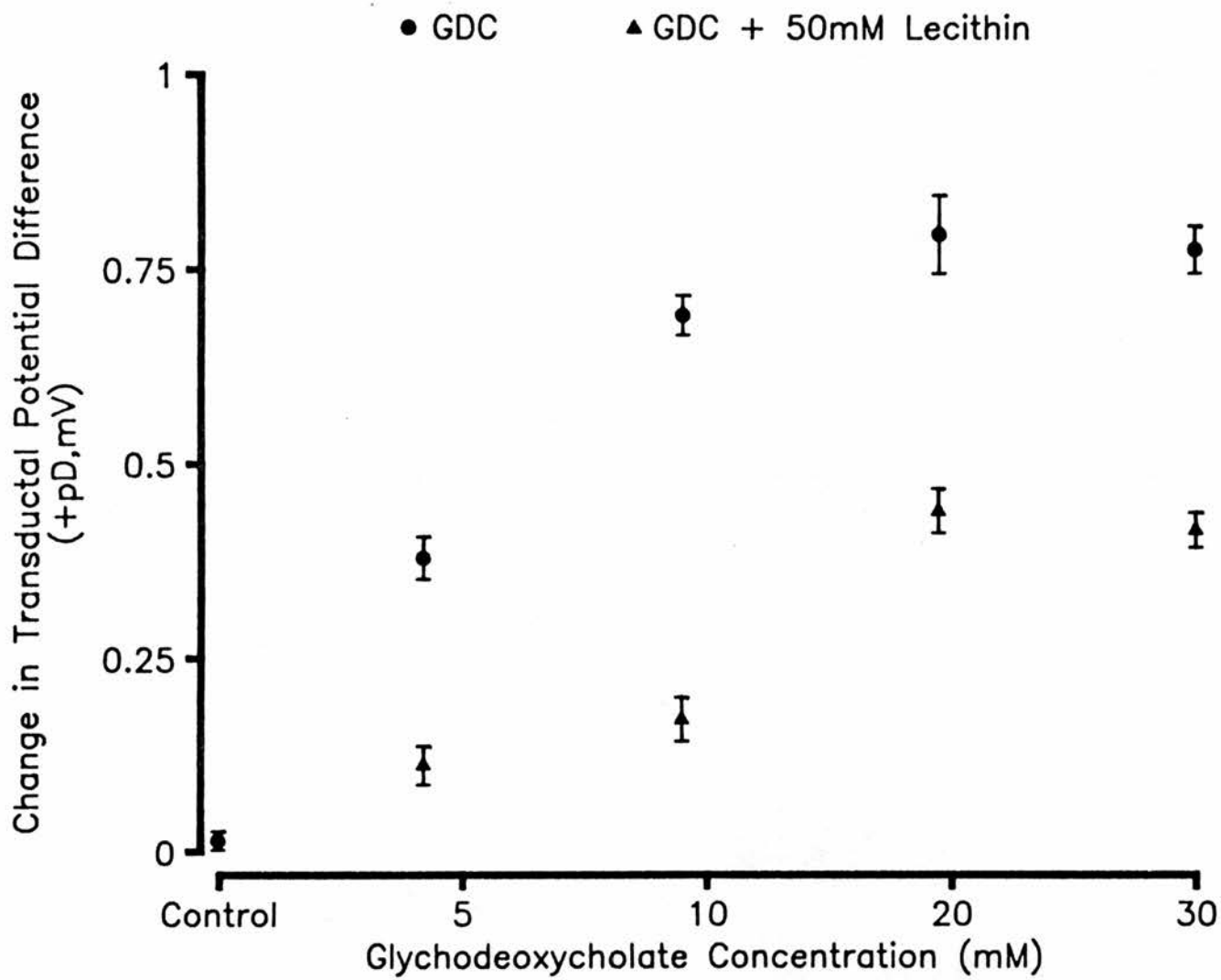


Fig. 39C GDC and lecithin vs. potential difference.

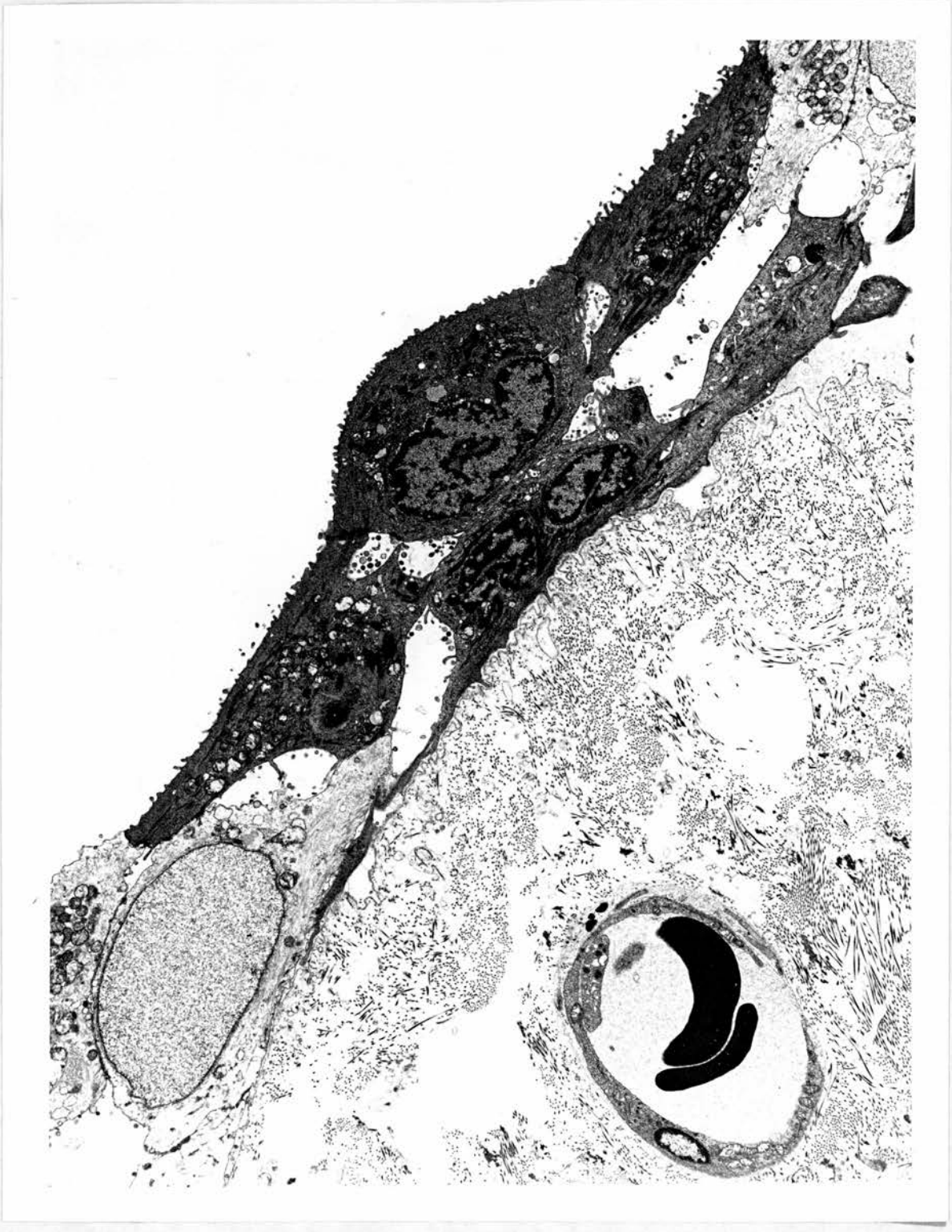


Fig. 40A Electron microscopy after perfusion of duct with GDC (x 5000).

Note cell death and cell shedding.

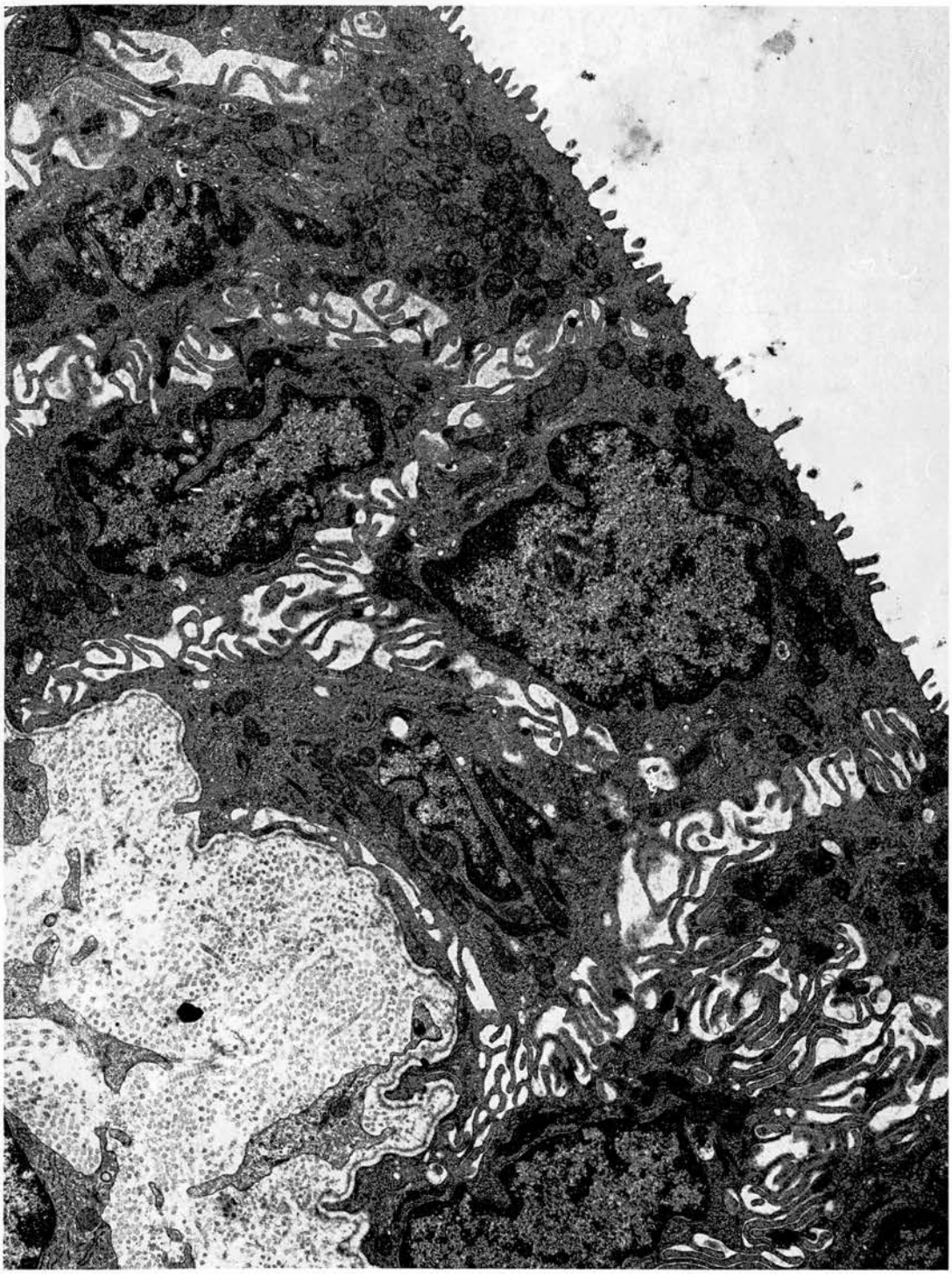


Fig. 40B Electron microscopy of duct after perfusion with GDC and lecithin.

Note normal cell appearance. Only abnormality is widening of intercellular spaces.

Fig. 40C Electron microscopy after perfusion of duct with GDC 10mM and lecithin (x 11250).

Note normal cells. Only abnormality is slight widening of intercellular spaces.

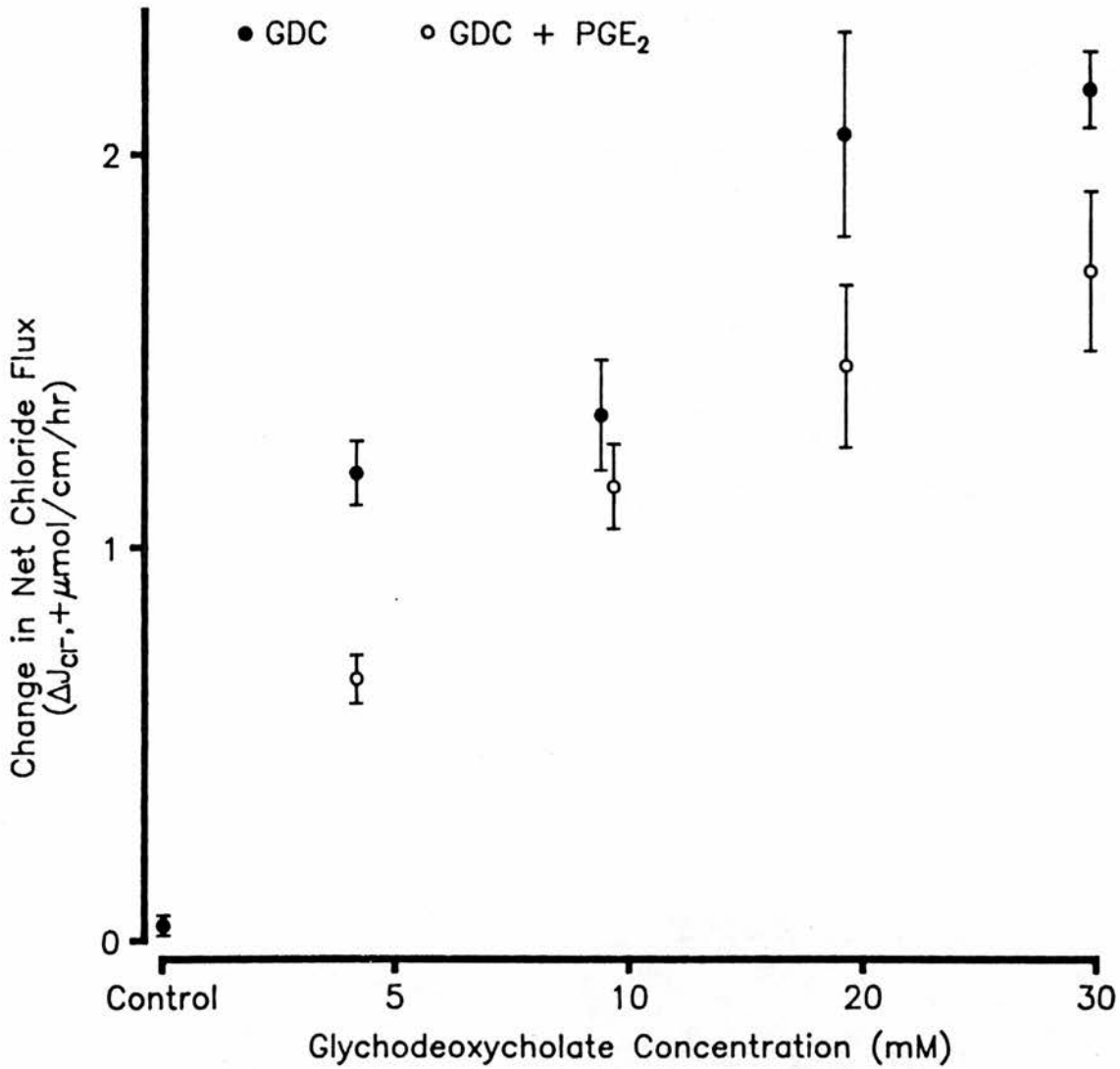


Fig. 41A Prostaglandin E_2 and GDC vs. chloride flux.

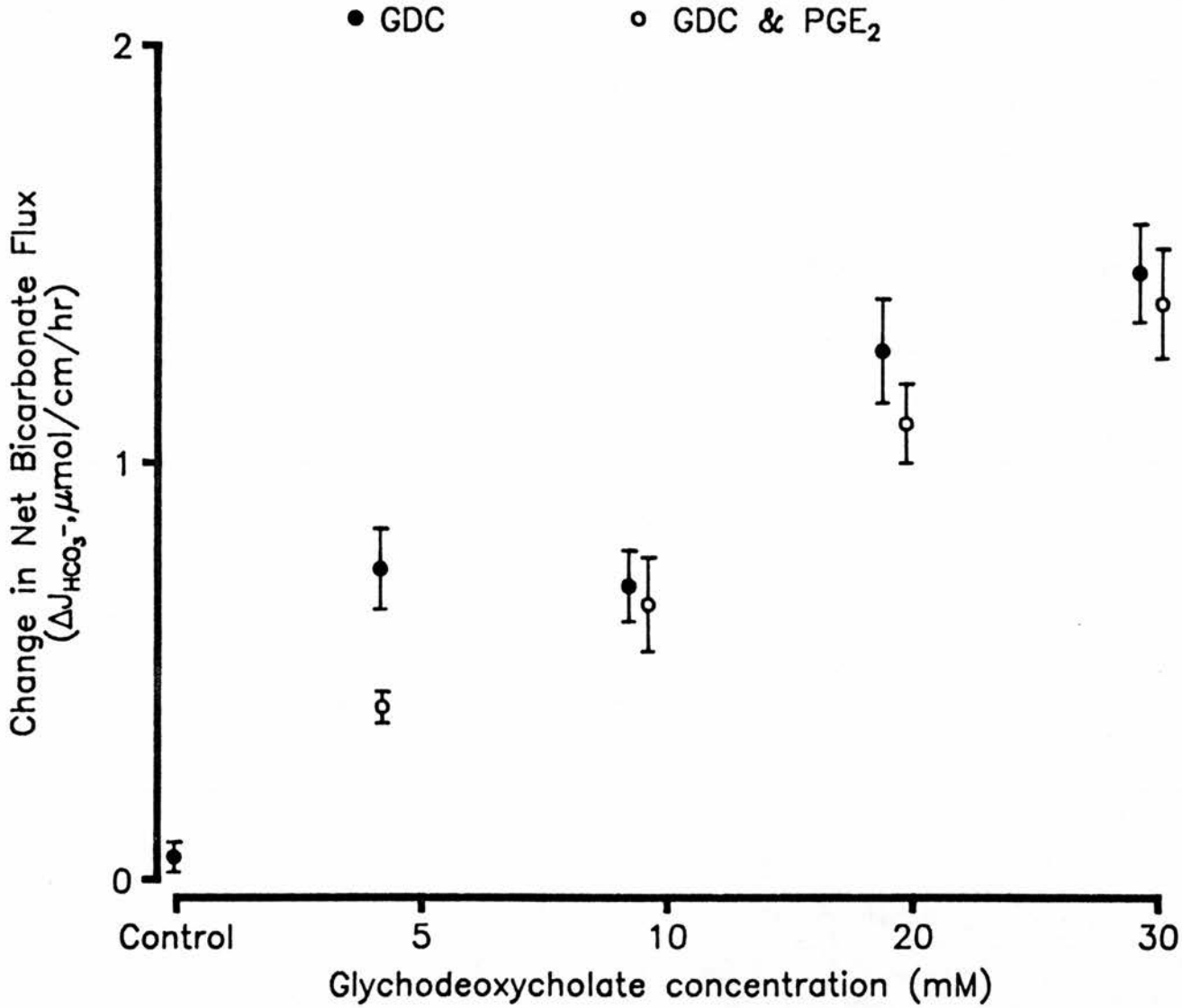


Fig. 41B Prostaglandin E_2 and GDC vs. Bicarbonate flux.

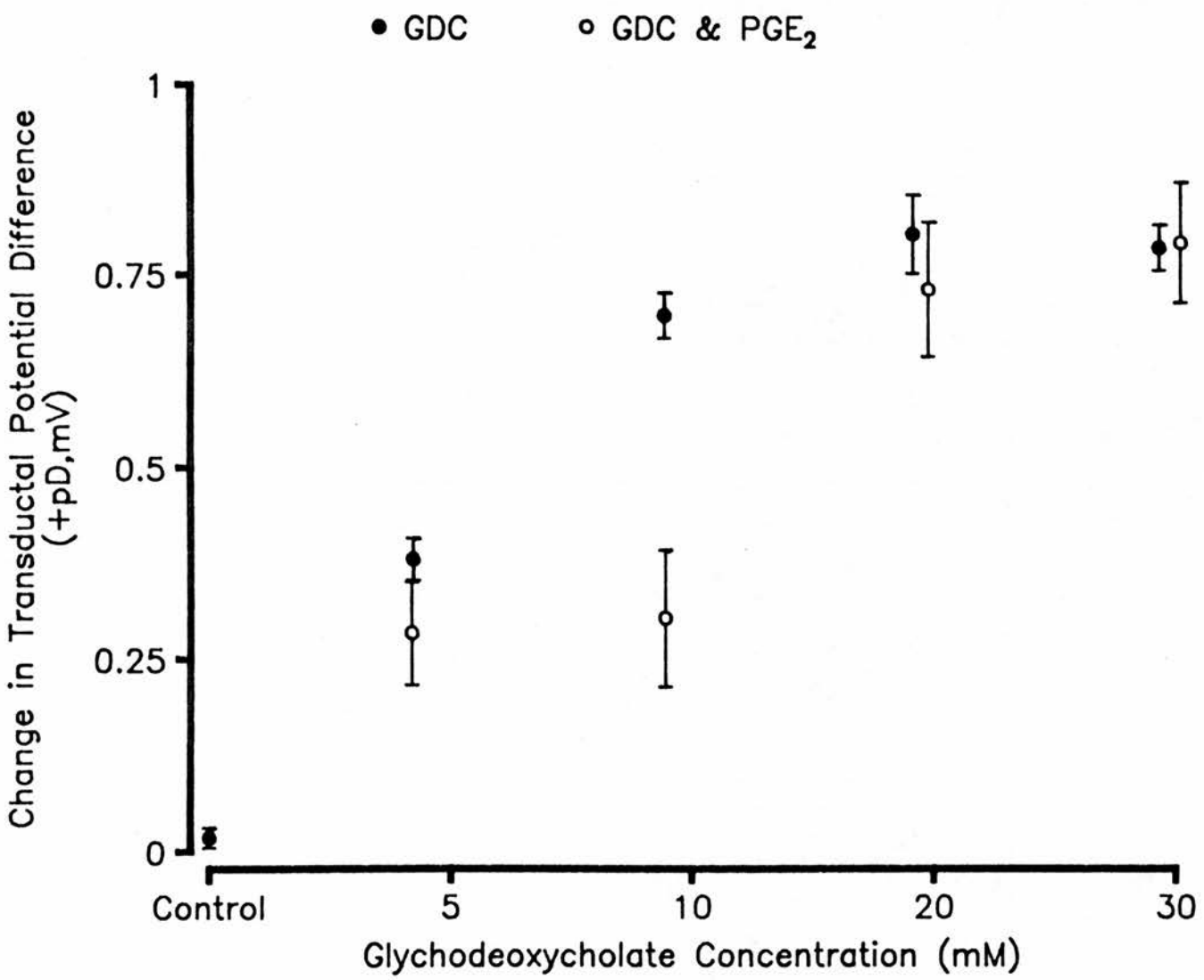


Fig. 41C Prostaglandin E_2 and GDC vs. pD.

Discussion

The bile-pancreatic duct of the rat may possess a mucosal barrier that normally impairs free diffusion of anions and macromolecules and this barrier appears to be important in maintaining duct integrity. I have previously shown that the mucosal barrier is broken by a variety of agents including sterile bile, infected bile, "pancreatitic" bile, bile salts, phospholipase A_2 and lysolecithin. The present results indicate that both prostaglandin E_2 and lecithin protect the barrier against damage by varying toxic agents. In this respect the pancreatic duct mucosal barrier is similar to the gastric mucosal barrier where "cytoprotection" is afforded by both PGE_2 (Robert 1979) and lecithin (Marriott 1984).

Administration of prostaglandin E_2 by way of intraductal pretreatment had a varying and incomplete protective action. PGE_2 alone produced no change in the duct epithelium. Whereas PGE_2 appeared to give excellent protection against 5 mM glycodeoxycholate induced barrier damage, there was an inconsistent and only partial effect on the damage produced by higher concentrations of bile salts. Indeed PGE_2 had virtually no effect on the mucosal damage produced by 30 mM glycodeoxycholate as mucosal ultrastructure still showed epithelial disruption and anionic flux and pD were little changed. Comparison of these results with the earlier studies of Reber and colleagues (1981a, Tweedie 1981, Mosley 1981) is difficult because of the many experimental differences. They used the cat pancreatic duct, perfused 16, 16 $DMPGE_2$ and studied mucosal damage induced by acidic aspirin or 15 mM glycodeoxycholate. A closer analysis of their results indicates that mucosal protection was only afforded at the very high intravenous dose of 50 µg/kg/hr of

16, 16 $DMPGE_2$; a value far in excess of the effective activity of 100 µg/kg/hr PGE_2 used in this study (16, 16 $DMPGE_2$ is five times as active as PGE_2). Furthermore these authors only examined the ultrastructural protection afforded by prostaglandin (50 µg/kg/hr) on 15 mM glycodeoxycholate-induced damage (Reber 1981a). The present study is comparable to that reported by Olazabal (1983a), where he found that prostaglandins I_2 and E_2 had little effect on deoxycholic acid-induced damage to the rat bile-pancreatic duct. However, Olazabal only partially measured duct integrity and this experiment has shown that a full assessment of duct integrity is necessary before definitive answers can be given with confidence.

Prostaglandins in physiological doses only protected the duct epithelium against reversible damage induced by low concentration of bile salt. The widespread epithelial disruption produced by high bile salt concentrations was relatively unaffected. Endogenous prostaglandin present in the bile or duodenum may have a physiological protective role when low bile salt concentrations are present. Whilst PGE_2 has been shown to have definite gastric cytoprotective properties, its role in mucosal protection of the pancreatic and bile ducts must remain debateable.

In contrast to the poor mucosal protection afforded by PGE_2 was that of lecithin. Lecithin was tested for its efficacy in preventing both bile salt-and lysolecithin-induced damage to the duct epithelium. Lecithin alone did not affect duct integrity demonstrating that by itself lecithin is relatively inert to cell membranes. Barrier damage produced by glycodeoxycholate was markedly reduced by the addition of lecithin. Whereas PGE_2 was only effective against the 5 mM concentration,

lecithin protected against all concentrations of glycodeoxycholate. Even with the 30 mM glycodeoxycholate concentration, lecithin produced a significant reduction in permeability change of 50 to 60%. Ultrastructural examination afforded a morphological correlation of this protective action. Lecithin reduced both the reversible appearances of cell swelling, widened intercellular spaces and cell flattening and the irreversible appearances of epithelial disruption and cell shedding. Thus, lecithin gave almost complete protection against glycodeoxycholate (5-30 mM) induced damage. There are several possible explanations for this action. Firstly, lecithin combines with bile salts to form mixed micelles which are less toxic to cell membranes. As approximately 2 molecules of lecithin are solubilized by each molecule of bile salt this would explain the ability of 50 mM lecithin to protect against up to 30 mM glycodeoxycholate damage. Secondly, lecithin may have a direct cytoprotective action on biliary, pancreatic and intestinal epithelium (Heuman 1980, Martin 1981). Thirdly, lecithin may protect against the direct detergent activity of bile salts. Previous workers have demonstrated that lecithin is protective against bile salt-induced damage to both the gastric (Marriott 1984) and biliary (Heuman 1980) epithelia. This study has shown that this protective activity also exists for the rat bile-pancreatic duct and probably therefore the pancreatic duct itself.

We have previously shown that phospholipase A_2 and lysolecithin have a pronounced barrier breaking effect. In this study lecithin reduced their effect on all indices of barrier damage and prevented cell vacuolation and epithelial disruption. As 1 molecule of lecithin can solubilize up to 10 molecules of lysolecithin, the lysolecithin:lecithin ratio may be important in determining bile toxicity. A 50 mM lecithin solution pre-

vented damage induced by 1% lysolecithin. Martin and Marriott (1981) have previously commented on the protective action of lecithin to lysolecithin induced damage of biological membranes. They have hypothesized that lecithin has a physiological action in protecting the intestinal tract from both bile salt and lysolecithin induced injury. This protective action might be compromised with bile reflux into the stomach which allows lysolecithin and bile salts to directly attack the gastric epithelium. It is of interest that Poncelet and Thompson (1972) have shown that the addition of lecithin to lysolecithin prevented the development of experimental acute pancreatitis.

These observations on the protective quality of lecithin may explain why both bile salts and lysolecithin alone have a much greater toxicity than whole bile, although both are present in equal concentrations in that bile. In whole bile lecithin is closely associated with both lysolecithin and bile salts, thus reducing their detergent properties. The presence of lecithin becomes, therefore, of utmost biological importance, because it protects biliary, pancreatic and intestinal mucosa from the potentially devastating effects of bile salts and lysolecithin. Lecithin itself appears to be more important in the physiological protection of intestinal cell membranes than endogenous prostaglandins. The possibility that other molecules are involved in pancreatic cyto-protection remains open to question. Two ratios involving lecithin may affect the toxicity of bile

(i) lecithin : bile salt

(ii) lecithin : lysolecithin

A reduction in these ratios will increase bile toxicity e.g. by either

a reduced lecithin level or by increased levels of toxic bile salts and increased lysolecithin as in infected bile. The level of lecithin in the bile of patients with pancreatitis is unknown. It is tempting to speculate that "pancreatitic" bile has a reduced lecithin level with an associated increase in toxic bile salts and lysolecithin. Indeed work on this question is now in progress in our laboratory.

Conclusions

1. Prostaglandin E_2 (100 µg/kg/hr) was partially protective against bile salt-induced damage to the bile-pancreatic duct and this protection was only strong when a 5 mM bile salt solution was used. The physiological role of endogenous prostaglandins in mucosal protection in the biliary and pancreatic ducts remains debateable.
2. Lecithin (50 mM) completely protected against bile salt, phospholipase A_2 and lysolecithin induced damage, possibly by mixed micelle formation.
3. Lecithin levels in bile may determine its pathogenicity with a reduced level increasing biliary toxicity. In particular the lecithin:bile salt and :lysolecithin ratios appear to be important.
4. Analysis of "pancreatitic" bile with regard to the relative levels of lecithin, bile salt and lysolecithin may hold the key to a further understanding of the initiation of acute gallstone pancreatitis.

PART III

THE BILIARY TRACT IN PATIENTS WITH ACUTE GALLSTONE PANCREATITIS

CHAPTER XIII

THE BILIARY TRACT IN PATIENTS WITH ACUTE GALLSTONE PANCREATITIS

Acute gallstone pancreatitis (AGP) is a common disease with a 10-15% mortality rate (Carter 1983). Opie in 1901 described a patient with fatal haemorrhagic pancreatitis who had a gallstone impacted at the sphincter of Oddi in the presence of an anatomical common channel. Since this description the concept of bile reflux into the pancreatic duct, after gallstone impaction at the ampulla of Vater, has experienced mixed fortunes. Most clinicians today, however, recognise that reflux of bile and/or duodenal contents is of cardinal importance in the pathogenesis of acute pancreatitis. Until recently several criticisms of bile reflux into the pancreatic duct remained unanswered;

(i) ampullary stones are present in only 5-7% of cases coming to surgery,

(ii) patients with jaundice resulting from impaction of gallstones at the ampulla do not necessarily develop acute pancreatitis,

and (iii) pancreatitis is only found in 3.6 - 6.5% of patients undergoing surgery for gallstones (Ranson 1979).

A major obstacle to acceptance of the hypothesis of gallstone impaction in the genesis of acute pancreatitis has been the observation that only about 10-20% of patients who have had attacks of pancreatitis prove to have gallstones in the common bile duct at the time of biliary surgery. For example, Goebell and Hotz (1979) in a review of some 1450 collected cases of gallstone pancreatitis found that gallstones were present in

the gallbladder alone in 72% of patients, in the common bile duct in 20%, and impacted at the ampulla of vater in only 2%. These observations prompted Acosta (1974) and Kelly (1976) to develop the theory of gallstone migration as a cause of acute pancreatitis.

Gallstone migration

Acosta in 1974 reported a classic study on acute gallstone pancreatitis. This was indeed pioneering work and perhaps the most significant advance in gallstone pancreatitis ideology since the initial observations of Opie in 1901. Acosta and Ledesma (1974) screened the stools of 36 patients with acute gallstone pancreatitis (AGP) by sieving all the faeces for 10 days after the attack using a 1 mm^2 pore filter. A parallel control series of patients with gallstones but without acute pancreatitis were similarly evaluated;

	presence of gallstones in the faeces	
pancreatitis (36)	34/36 (94%)	$P < 0.001$
No pancreatitis (36)	3/36 (8%)	

(Acosta and Ledesma, 1974)

A single stone was found in 24 patients, and two or more stones (up to 80) were identified in the remaining 10 patients. The patients who had only one attack of pancreatitis usually eliminated a single stone, whereas the finding of several stones was frequently associated with recurrent attacks of pancreatitis. The size of the calculi found in the stools ranged from 1 to 15 mm and in every case the stones in the faeces were identical, both physically and chemically, to those later removed from the gallbladder. Acosta and Ledesma concluded: "the temporal sequence between pancreatic crisis and the appearance of stones

in the faeces strongly suggests that acute pancreatitis developed in these patients as a result of an ephemeral mechanical blockage of the ampulla that, in turn, was caused by migrating stones. The finding of small-sized stones in the faeces, in association with an apparently functioning gallbladder containing identical stones, identifies the gallbladder as a major source of migrating stones. The finding of stones in the faeces of only three control cases suggests that calculi seldom migrate without producing acute pancreatitis".

Kelly in 1976 repeated Acosta's earlier work. He studied 45 patients with AGP and a similar control group;

	presence of gallstones in the faeces		
pancreatitis (45)	38/45	(84%)	
			$P < 0.001$
No pancreatitis (45)	5/45	(11%)	

(Kelly 1976)

One stone was passed by 27 patients and two to 25 stones by the remaining 11 patients. The stones ranged from one to 12 mm in size. Each individual's calculi, found in the stool and at operation, were similar grossly and identical chemically. He later reported on an increased number of patients (Kelly 1980) with AGP and found stones in the faeces of 123/143 patients (86%).

These observations stimulated the development of the "migratory stone" concept; i.e. pancreatitis develops when a stone migrates from the gallbladder and impacts temporarily at the ampulla of Vater, causing reflux into the pancreas. The stone then passes through the ampulla and continues its journey into the gastrointestinal tract. Failure to recover

stones from the faeces in 6-15% of patients with AGP may be due to

(i) the stone remains embedded in the ampulla or refluxes into the common bile duct. This may explain why only 5-8% of stones are found impacted at the time of operation.

(ii) stones smaller than 1mm in diameter, and thus not detected in the faeces, may be responsible.

More recently, Mayer and McMahon (1983) from Leeds have found stones in the faeces in 35/43 patients (81%) of patients following an attack of AGP. Very few of the stones retrieved were above 5 mm in diameter.

The concept of gallstone migration is of paramount importance in the understanding of AGP. Small stones in the gallbladder migrate through the ampulla of Vater and into the gut. A summary of collected figures on stone retrieval gives the following results;

	stones in faeces	
pancreatitis	192/222 (86.5%)	$P < 0.001$
No pancreatitis	8/81 (10%)	

(Acosta 1974, Kelly 1976, Kelly 1980, Mayer 1983)

Recurrent gallstone pancreatitis

Further evidence for gallstone migration being responsible for AGP has come from Kelly (1982). He studied 35 patients who had previous surgery for gallstone pancreatitis involving cholecystectomy and removal of gallstones (1-12 years previously). All 35 patients presented with recurrent stones in the common bile duct and 92% had a recurrent attack of pancreatitis. Interestingly recovery of stones from the faeces in this group of patients was only 37%, although these recovered stones were all small (< 8 mm) suggesting that bigger stones were retained in

the common bile duct. This study emphasized the importance of complete operative clearance of the biliary tree of stones. Gallstones passing through the ampulla of Vater are mechanical initiators of inflammation and removal of all stones obviates recurrent attacks of AGP. If stones recur or are retained then further attacks of AGP are to be expected as the same anatomical and physiological arrangements in the bile and pancreatic ducts are present. In patients with pancreatitis and cholelithiasis, surgical treatment of biliary disease reduces the risk of recurrent pancreatitis from 36-63% to 2-8% (Ranson 1979).

Timing of surgery in pancreatitis

If migration of gallstones is truely the cause of AGP then earlier operations should be associated with more stones being found in the common bile duct. Conversely if one operates later, as was until recently standard surgical practice, then far fewer stones are present as most have migrated into the duodenum.

Acosta and colleagues (1978) studied a group of patients with AGP, in whom surgery was performed within 48 hours of the onset of symptoms. In the 46 patients undergoing early surgery all had macroscopic evidence of acute pancreatitis. Choledocholithiasis was present in 38/46 patients (83%) and in 33 (72%) a gallstone was impacted at the ampulla. An update on 78 patients was reported by Acosta and associates in 1980 when they found the time of surgery after the onset of AGP to be closely related to biliary pathology;

time of operation (days)	Number of patients	Number with Ampullary stones
0-2	55	41/55 (75%)
3-4	11	5/11 (45%)
>5	12	3/12 (25%)

(Acosta 1980)

The stones removed from the ampulla were small, ranging from 2 to 6 mm in diameter, and in each case the similarity between the stones removed from the gallbladder identified the latter as the major source of migrating stones.

Kelly (1980) in the same year examined the findings at surgery in 24 patients with AGP who underwent surgery within 48 hours of an acute attack. Of these 24 patients, choledocholithiasis was present in 17 and 15 (63%) had impacted ampullary stones. 132 patients underwent surgery later in the same admission at 5-8 days. In this group only 5 patients had evidence of ampullary stones. These results are summarized below;

time of operation (days)	Number with Ampullary stones (%)	Number with faecal stones (%)
0-3	63%	-
5-8	5%	83%

(Kelly 1980)

By delaying surgery most patients resolve their pancreatitis by endogenous disimpaction of the ampullary stones into the gut.

Stone and co-workers (1981) reported a controlled trial in which 65 patients with AGP were randomly selected for early (within 73 hours) or late (3 months) biliary operations. At early operation (36 patients) pancreatitis was in the acute oedematous form in 29 patients, necrotizing in six and haemorrhagic in one. Acute inflammatory changes were seen in three patients undergoing late surgery. The locations of the gallstones at the time of operation were;

location of gallstones	Early operation	Late operation	
gallbladder	97%	100%	
common duct	75%	28%	$P < 0.02$
duodenum	31%	0%	$P < 0.01$

(Stone 1981)

The distal choledochus and ampulla were inflamed in 89% of the patients who underwent early operations, but in only 17% operated upon electively ($P < 0.01$). Concomitant acute cholecystitis was present in 31% of patients where surgery was performed early and in only 3% of late operation. Stone and colleagues also remarked on the sudden "gush" of pancreatic juice when the ampulla was opened. This data further supports the concept that biliary pancreatitis is probably initiated by gallstone passage through, or lodgment at, the ampulla of Vater. The resultant ampullary oedema with or without gallstone impaction appears to be the anatomic cause for obstruction and major pancreatic duct reflux with subsequent pancreatitis. It is of interest that relief of the ampullary obstruction produced early recovery from acute pancreatitis.

Further evidence on stone passage down the biliary tree has been afforded by Paloyan (1975) Osborne (1981) and Carter (1983a,b). These authors commented on the increased incidence of choledochal and ampullary stones the earlier surgery was undertaken during AGP. The advocates of surgery during the same admission in AGP point to the high incidence of recurrent pancreatitis whilst the patient is awaiting elective admission. Whilst these patients still have stones in the gallbladder and biliary tree there is a high chance of stone migration causing further attacks of pancreatic inflammation.

Recently Safrany and Cotton (1981) and Rosseland (1984) performed early ERCP and sphincterotomy for acute gallstone pancreatitis. They noted the high incidence of oedema of the ampulla and the large number of impacted stones that could be directly visualized. Endoscopic removal of the stones resulted in a rapid reduction of pancreatic inflammation. These reports, although containing small numbers of patients, serve to reinforce the concept of gallstone migration.

If biliary surgery is performed at a later date after an attack of AGP (>6 weeks) then the incidence of choledocholithiasis is similar to that of patients without a previous pancreatic history. Taylor and associates (1983) have recently studied factors that are associated with the presence of common bile duct stones. They found that a past history of acute gallstone pancreatitis (>6 weeks) was not associated with an increased incidence of choledocholithiasis. As the majority of gallstones producing AGP are small and migrate through the ampulla within a few days this finding is not surprising. It does, however, explain the low incidence of ampullary stones in patients undergoing elective surgery after an attack of AGP.

Gallstones in acute gallstone pancreatitis

Many surgeons have commented on the small size and high number of gallbladder stones in patients with AGP. Acosta (1974) and Kelly (1976) have shown that stones recovered from the faeces, and thus responsible for initiating the pancreatic inflammation, are small and generally less than 5-8 mm in diameter. This measurement might, however, be self selective as the larger stones are retained in the common bile duct. Both Acosta (1974, 1980) and Kelly (1976, 1980) .. stress that those

patients with AGP have small stones in the gallbladder which are similar to those found in the faeces. Early operative intervention has demonstrated that impacted ampullary stones are small (2-6 mm) and in those patients with associated pancreatic duct reflux the stones are even smaller (<3 mm) (Acosta 1980). The chemical nature of gallstones inducing AGP has been studied only briefly. Acosta (1974) demonstrated a chemical makeup similar to that of gallstones in patients without pancreatic inflammation. Kelly (1976) found 82% of the gallstones to be cholesterol, 15% to be pigmented and one patient to have calcium bilirubinate stones, implying that the chemical nature of gallstones is not different in patients with AGP.

McMahon and Shefta (1980) examined the physical characteristics of gallstones in patients with acute pancreatitis with the results shown below;

	patients with pancreatitis (18)		patients with no pancreatitis (28)
Number of gallbladder stones	119±173	NS	84±178
Number with >5 gallbladder stones	22%		39%
weight stones (g)	0.31±0.7	P<0.02	0.74±0.9
types of stones			
small irregular	78%	P<0.05	43%
large rounded	22%		57%

(McMahon 1980)

The gallstones associated with AGP are smaller, more numerous, lighter and more irregular than those found in control patients. All these physical characteristics fit with the concept of gallstone migration and temporary ampullary impaction.

Cystic duct diameter and flow

McMahon and Shefta (1980) have produced the only data available on the size of the cystic duct in patients with AGP. They did not measure the width of the duct directly but instead employed the ingenious technique of flow through the cystic duct at a constant head of pressure over a given time. As patients with pancreatitis transmitted significantly larger volumes than controls (282±196 ml vs 134±168 ml, $P<0.01$) the cystic duct of patients with gallstone pancreatitis was larger than that of controls without AGP. The functional calibre of the cystic duct may be important in facilitating transfer of gallstones from the gallbladder into the common bile duct . As flow through a tube is highly dependent on the radius of that tube, patients with AGP also have a wider cystic duct than that of controls. There has not, however, to my knowledge been any study of the actual diameter of the cystic duct in vivo.

Common bile duct diameter and pancreatitis

The size of the common bile duct is a valuable clue to the presence of biliary obstruction as patients with stones either present or recently passed from the common bile duct tend to have larger ducts. In the context of gallstone migration from the gallbladder down the common bile duct and through the ampulla a degree of choledochal and pancreatic duct obstruction must occur in patients with pancreatitis. Osborne and colleagues (1983) analyzed the operative cholangiograms of patients undergoing biliary operation following an attack of AGP. They measured common bile duct diameter in the presence and absence of choledocholithiasis thus;

	Mean common bile duct diameter (mm)		
	pancreatitis	No pancreatitis	
CBD stones present	11.2±2.6	12.3±2.5	NS
CBD stones absent	10.5±3.2	8.3±2.5	P < 0.001
	NS	P < 0.001	

(Osborne 1983)

A greater mean common bile duct diameter was also seen in patients undergoing surgery within 4 weeks of the attack of acute pancreatitis (11.5±3.1 mm) than in those who had delayed surgery (9.2±2.5, $P < 0.05$). These results indicate that firstly the common bile duct is wider in patients after an attack of AGP despite the absence of choledochal stones, and secondly the width of the common bile duct is greater the earlier surgery is performed after AGP. This simple study supports the concept of gallstone migration with temporary obstruction of the common bile duct.

Csendes (1979) measured the size of the common bile duct at ERCP examination and found the mean size in controls to be 6.0±1.5 mm. In those patients with gallbladder stones alone the diameter of the common bile duct was 10.3±0.8 mm and in those patients with choledochal stones the size was 16.3±1.3 mm.

It remains uncertain, however, whether the increased size of the common bile duct in AGP is primary, thus facilitating stone migration, or secondary to temporary obstruction. Moreover the relationship between common bile duct diameter, cystic duct diameter and the number and size of gallstones has yet to be clarified.

Pancreatic Duct Reflux (PDR)

PDR is commonly observed at operative cholangiography, but its

significance is uncertain. Schulenberg (1966) suggested that it had no clinical importance whereas Cuschieri (1973) found that 27% of pancreatic ducts into which reflux occurred were abnormal. The reported incidence in patients undergoing biliary surgery varies from 8 to 35 per cent (Cuschieri 1973, Taylor 1980, Ivy 1952, Schulenberg 1966). The first study of note was reported by Howell and Bergh (1950) who studied PDR by injecting contrast media down a T-tube. In 18 patients with previous pancreatitis PDR was seen in 14 (78%) whereas PDR was present in only 13/47 (28%) of patients with biliary disease alone. Most cases showing PDR developed an increase in amylase levels indicating that reflux might be of significance. They further injected bile into patients with PDR and determined a marked rise in amylase in all of these (this study would now be considered unethical).

Cuschieri and Hughes (1972, 1973) performed an elegant series of experiments on PDR using closely controlled conditions. They studied 211 patients and found pancreatic duct reflux in 31 cases (15.4%). Eight of the 31 pancreatograms showed an abnormal pancreatic duct with dilatation being the most obvious abnormality. There was no relationship between PDR and the presence of choledochal stones; PDR was seen in 11% with stones and in 15% without. Reflux of contrast medium into the pancreatic duct was associated with a low resting choledochal pressure (mean 2.3 mm, range 0-6 mm Hg). From these findings the authors concluded that PDR was not associated with either obstructive pathology of the lower choledochus or biliary hypertension.

Since the reports of Cuschieri and Hughes there have been several references to the incidence of PDR during operative cholangiography in

relation to previous gallstone pancreatitis. Kelly (1976) found PDR in 67% of patients with gallstone pancreatitis in contrast to only 18% in a control group. Later the same author (Kelly 1980) found PDR to occur in 87% of patients with recurrent pancreatitis. Acosta (1980), in contrast, demonstrated a lower incidence of PDR in the acute pancreatitis situation. This study was not, however, comparable to the other reports as the operation was carried out within 48 hours of the attack of AGP and stones were frequently found blocking the ampulla.

Schein and Beneventano (1968) found PDR in 9 of 20 patients when contrast was infused down a T-tube. They found the average pressure required for pancreatic duct filling was 23 cm H_2O and there was no correlation between the length of duct visualized and the filling pressure. Importantly pancreatic duct filling was painless. Further reports by Carey (1975) and Thurston (1975) have emphasized the increased incidence of PDR in patients with pancreatitis, although it would appear that PDR is a normal harmless event in some patients.

Taylor and Rimmer (1980) have recently commented on the high incidence of PDR in patients with pancreatitis. They studied the operative cholangiograms of 292 consecutive patients undergoing biliary surgery with the findings summarized below;

	Number with PDR	(%)	
Total	56/292	- 19.2%	
pancreatitis	11/21	- 52.4%	$P < 0.001$
No pancreatitis	45/271	- 16.6%	

(Taylor 1980)

There was no relationship between PDR and choledocholithiasis, jaundice, bile duct diameter and nor could the length of reflux be related to acute gallstone pancreatitis. Interestingly more men had evidence of PDR in relation to previous pancreatitis. This study demonstrated an increased predisposition to the reflux of biliary contents into the pancreatic duct, irrespective of choledochal pathology, in patients with previous pancreatitis. This association between acute gallstone pancreatitis and PDR has since been confirmed by Osborne and colleagues (1983) who described PDR in 21/49 patients with pancreatitis in contrast to only 11/50 of controls.

Thomas and co-workers (1983) recently reported briefly on the relationship between PDR and serum amylase levels. They could find no correlation between PDR and an elevated serum amylase, and found that hyperamylasemia more commonly followed surgical trauma to the sphincter of Oddi. These authors also suggested that the presence of pancreatic duct filling during cholangiography probably underestimates the frequency with which pancreatic duct reflux can occur.

Reports of PDR seen on operative cholangiography can be summarized as:

(i) PDR occurs in some patients without pancreatic disease.

(ii) patients with a history of AGP have a much higher incidence of PDR than those with gallstones alone.

(iii) PDR is not related to choledochal stones.

The overall incidence of PDR is 15-35%. In patients without pancreatic disease it is 15-20% and in those with previous pancreatitis 55-65% ($P < 0.001$).

There have not to my knowledge been reports of the relationship between pancreatic duct reflux and the size of the pancreatic duct, the size of the common bile duct, the length of the common channel and the length of pancreatic duct filling.

Pancreatic Duct Diameter

The recent advent of ERCP examination has enabled the diameter of the pancreatic duct to be measured. Anacker (1977) has commented on the size of the normal pancreatic duct and has stated that the normal size in the head of the gland is 2-3 mm with a wide range of normality (1-6 mm). Furthermore the bile duct diameter is usually twice that of the pancreatic duct. Csendes and associates (1979) reported on the size of the pancreatic duct in relationship to common bile duct size and biliary pathology. Their figures (mean ± SD) are given below;

Patients	diameter of common bile duct (mm)	diameter of pancreatic duct (mm)
normals	6.0±1.5	2.0±0.2
stones in gallbladder	10.3±0.8	5.3±0.4
stones in common bile duct	16.3±1.3	5.3±0.4

(Csendes 1979)

The presence of stones in the biliary tract is thus associated with an increased diameter of the pancreatic duct.

Common Channel

Many studies have investigated the anatomy of the pancreatobiliary junction. Ivy (1952), in an exhaustive review of previously published reports, found that a functioning common channel was present in approximately 70% of specimens. Hand (1963), in an anatomical review of 30

papers covering 3,000 dissections of the ampulla of Vater, reported that a common channel was present in about 80% of the patients and in 56% of patients this common channel was 5 mm or less in length. Length of the common channel is important when considering possible PDR after temporary impaction of a small stone (often < 3 mm in diameter). Kelly (1976) reported on the relationship between gallstone pancreatitis, gallstone migration and a common channel. He found a functioning common channel in 79% of patients who had stones recovered from the faeces. He also made the interesting observation that a functioning common channel not only favoured reflux but also stone passage. A later report from Kelly (1982) demonstrated 87% of patients with AGP to have a functional common channel. Despite these observations there has been no reported details on the relationship between the length of the common channel, pancreatic duct reflux and gallstones.

Summary

A number of reports on varying aspects of the biliary tract in relationship to acute gallstone pancreatitis can be summarized as:

1. Acute pancreatitis occurs in only 3-7% of patients with gallstones.
2. Stones are found in the faeces of most patients after an attack of AGP. These stones tend to be small and derive from the gallbladder.
3. The earlier surgery is performed during AGP the more often are ampullary stones and oedema noted.
4. Delayed surgery (> 4 weeks) results in a low incidence of choledocholithiasis as the stones have passed into the gut by this time.

5. The gallstones in patients with AGP tend to be smaller, lighter, more irregular and more numerous than in control patients.
6. The cystic duct is wider in patients with AGP suggesting easier passage of gallstones.
7. The common bile duct is wider after an attack of AGP regardless of the presence of choledocholithiasis, suggesting temporary biliary obstruction by a migrating gallstone.
8. There is an increased incidence of PDR in patients with pancreatitis.
9. A functioning common channel is often found in association with PDR in patients with pancreatitis.
10. The theory of gallstone migration now appears well proven.

There is a need to measure all the biliary tract characteristics in patients with AGP, as to date authors have concentrated on various aspects of the biliary tract alone without attempting to correlate them.

Patients and Methods

Object:

To establish the characteristics of the biliary tract in patients with gallstone pancreatitis as compared with those patients without pancreatic disease (controls).

Acute gallstone pancreatitis:

This was defined as acute pancreatitis initiated by gallstones i.e.

(i) No history of alcohol or other predisposing cause.

(ii) Proven gallstones at operation.

(iii) Proven acute pancreatitis by - operation.

or - clinical picture, with raised serum amylase.

Operations were carried out at one day to 5 weeks after the attack of pancreatitis. During the last 2 years of the study there was an increasing tendency to operate earlier in the recovery period and within the same admission. This difference in the operative policy will be later discussed in relation to biliary pathology.

Patients

The series consisted of 664 consecutive patients undergoing cholecystectomy in the Department of Surgical Gastroenterology, Royal Infirmary, Manchester during the period 1978 to 1983. All were studied prospectively with a post-operative follow up of 9 months to 5 years (most greater than 18 months). Ten patients died in the post-operative period giving a mortality rate of 1.5%.

Factors assessed

The following 21 factors were assessed in these patients (see later for individual details of each factor).

1. age
2. sex
3. jaundice (Bilirubin > 50 μmol/l; normal range 3-17)
4. operative findings vs time of operation
5. post-operative mortality
6. post-operative morbidity
7. number of gallbladder stones
8. size of gallbladder stones (mm), smallest and largest
9. diameter cystic duct (mm)
10. squeeze test (see later)
11. number of choledochal stones
12. size of choledochal stones (mm)
13. (i) diameter common bile duct; dilated or not
    (ii) size of common bile duct on cholangiography (mm)
14. duodenal filling on cholangiography
15. pancreatic duct reflux (PDR) on cholangiography.
    Where PDR was present several additional factors were measured.
    16. clinical features
    17. size pancreatic duct at maximum diameter (mm)
    18. angle of reflux (degrees), namely the angle between the bile and pancreatic ducts
    19. length of reflux down pancreatic duct (mm)
    20. length of common channel (mm)
21. long term follow up

The various measurements carried out are summarized in fig. 42.

Operative cholangiography

Hypaque (20%) was injected into the biliary tree through either direct needle puncture or via a cannula in the cystic duct. Three films were taken after injection of 3, 8 and 15 mls of contrast respectively. Measurements were performed on the first film.

Statistical methods

All variables were entered into a computer with the aid of the Department of Computation, University of Manchester. Statistical significance was assessed by either univariate or multivariate analysis. The following non-parametric statistical methods were used, with $P < 0.05$ being regarded as significant.

Chi-square (X^2), standard score (z value).

Rank sum tests - Wilcoxon, Spearman.

Fishers exact test for small numbers.

Mann-Whitney U test, student's t test.

Multiple logistic regression.

Values given are as median with range or mean $\pm$ standard deviation.

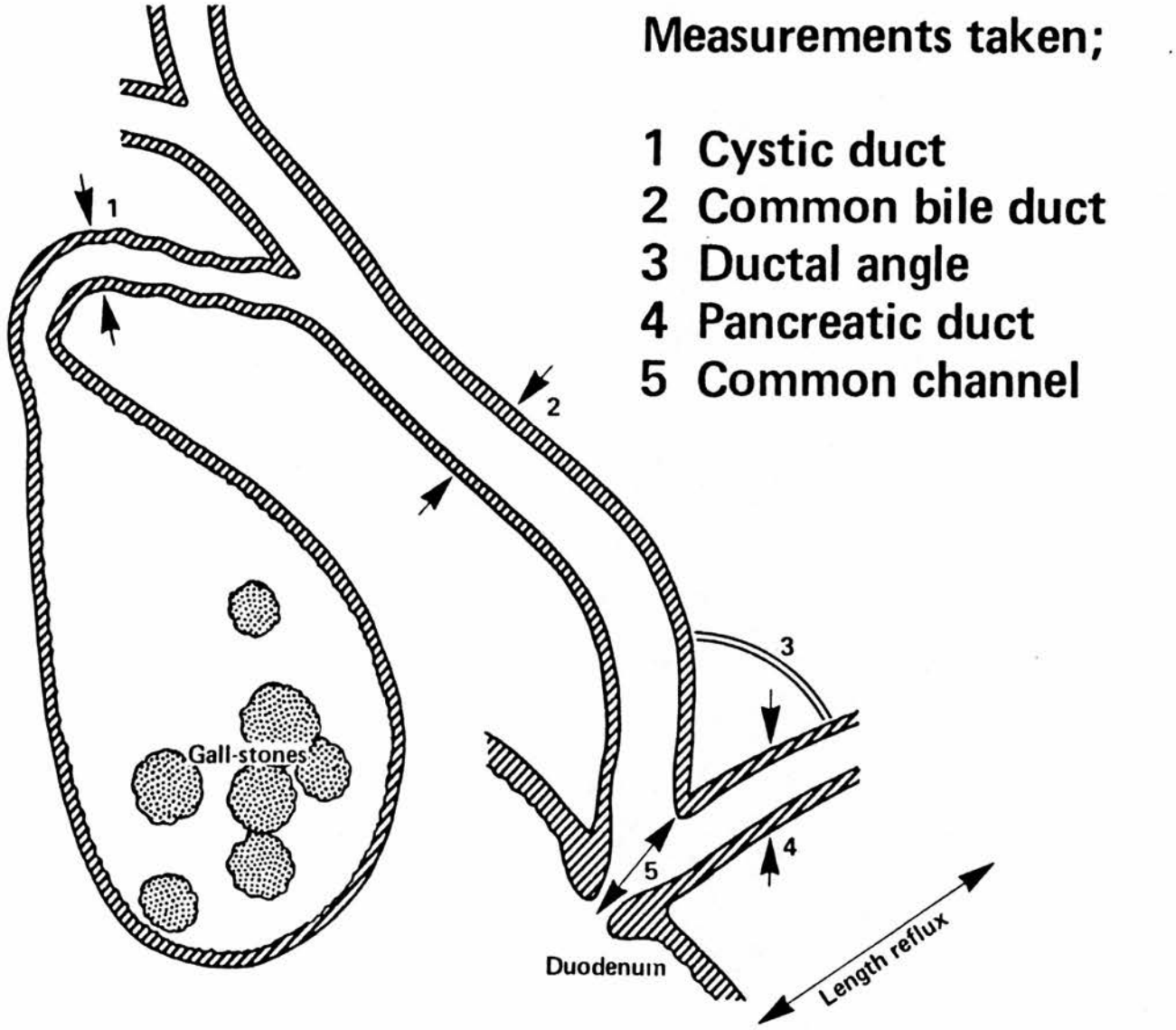


Fig. 42 Measurements of biliary tract.

Results

Pancreatitis

52 patients had a definite history of acute gallstone pancreatitis giving an overall incidence of 7.8% for patients with gallstones over the study period.

	No. of Patients	(%)
gallstone pancreatitis	52	(7.8%)
No pancreatitis (controls)	612	(92.2%)
Total	664	

1. Age (66 patients)

   There was no significant difference between the age of patients with and without AGP.

   pancreatitis median age 54.5 (range 23-76)

   No pancreatitis median age 51.7 (range 15-88)

   P = 0.23

2. Sex (66 patients)

   Of the 52 patients with AGP 25 were male and 27 female. A comparison of this ratio to that of control patients revealed that significantly more males with gallstones developed pancreatitis.

	pancreatitis	No pancreatitis	Total
male	25	149	174
female	27	460	487
	52	609	661

$X^2 = 12.6$, P = 0.0004

Of the patients who had previous pancreatitis 48% were male and 52% female. In contrast, of the control patients only 24.5% were male and 75.5% female.

3. Jaundice (664 patients)

There was no difference between the number of patients with and without AGP as regards the presence of pre-operative jaundice.

	pancreatitis	No pancreatitis	Total
jaundice	10	127	137
No jaundice	42	485	527
	52	612	664

$X^2 = 0.07$, $P > 0.1$

pancreatitis : 19.2% jaundiced, 80.8% not.

No pancreatitis : 20.7% jaundiced, 79.3% not.

4. Operative findings related to the time of operation (52 patients)

The time of operation after the initial attack of acute gallstone pancreatitis was arbitrarily divided into 0-2 days, 3-7 days, 8-21 days and 22 days. The figures for the number of patients and the incidence of choledochal stones are given below.

time (days)	Number of patients	gallbladder stones	choledochal stones	stones impacted at ampulla
0-2	3	3	2 (67%)	2 (67%)
3-7	8	8	4 (50%)	2 (50%)
8-21	17	17	4 (23.5%)	1 (6%)
> 22	24	24	3 (12.5%)	1 (4.2%)
overall	52	52(100%)	13(25%)	6 (11.5%)

Thus the earlier the operation the greater chance of finding stones both in the common bile duct and impacted at the ampulla of Vater.

5. Operative mortality rate (664 patients)

Although there was an increased mortality rate in patients undergoing operation for AGP (5.77% vs 1.14%; $X^2 = 6.91$, $P < 0.01$)

the two groups were not comparable as

(i) a number of patients underwent operation for failure of the pancreatitis to settle and were thus a high risk group.

(ii) more common bile duct exploration were carried out in patients with pancreatitis, with its attendent increased morbidity and mortality.

	pancreatitis	No pancreatitis	Total
recovered	49	605	654
died	3	7	10
	52	612	664

$X^2 = 6.91$, $P < 0.01$

The causes of death in the AGP group were; acute haemorrhagic pancreatitis with septicaemia, acute haemorrhagic pancreatitis with pancreatic abscess and renal failure. The causes of death in the patients without pancreatitis were cardio-respiratory problems (3), septicaemia (2), gastro-intestinal bleeding (1) and renal failure (1).

6. Postoperative morbidity (664 patients)

The only postoperative complication that was more common in patients with AGP was that of wound infection.

	pancreatitis	No pancreatitis	Total
wound infection	7	38	45
No wound infection	45	574	619
	52	612	664

$X^2 = 3.99$, $P < 0.05$

There was no difference in the incidence of wound dehiscence,

subphrenic abscess, respiratory and cardiac problems and retained stones between the two groups of patients. One patient in each group developed postoperative pancreatitis.

7. Number of gallbladder stones (664 patients) (fig. 43)

These were counted on opening the gallbladder at the end of the operation and scored 0 to 10 and multiple. Multiple ranged from 11 to 100's of small stones making an exact measurement of the number difficult and multiple was therefore arbitrarily coded as 30. Patients with AGP had more stones in their gallbladders, a result of marginal significance.

	pancreatitis	No pancreatitis
Number of patients	52	612
mean rank of number of gallstones	378.8	328.6
mean ± SD number of stones	21.46±13.11	16.69±14.31
median (range)	29.8 (1-30)	29.6 (1-30)

$Z = 1.9916$, $P = 0.0464$

8. Size of gallbladder stones (196 patients)

On removal of the gallstones the sizes of the smallest and largest stones were measured to the nearest mm by a caliper. The smallest measurement was used for study purposes as it is now well established that it is these small stones that are responsible for AGP.

Patients with AGP had smaller stones than those without (fig. 44).

	pancreatitis	No pancreatitis
Number of patients	22	174
mean rank of size of gallstones	59.73	103.4
mean ± SD diameter (mm)	2.68±3.43	4.64±4.10
median diameter (range)	1.7 (1-14)	3.14 (1-25)

Z = -3.448, P = 0.0006

There was no significant difference between the size of the largest gallstone: 7.05±3.56 vs 9.18±5.34 mm.

9. Diameter cystic duct (427 patients)

The diameter of the cystic duct close to its entrance to the common bile duct was measured carefully at operation to the nearest mm. Patients with AGP had a significantly larger cystic duct than those without pancreatic disease (fig. 45).

	pancreatitis	No pancreatitis
Number of patients	34	393
mean rank of cystic duct size	292.1	207.2
mean ± SD diameter (mm)	4.94±2.29	3.68±2.04
median diameter (range)	4.62 (1-12)	3.1 (2-18)

Z = 3.96, P = 0.001

10. "squeeze" test (178 patients)

At the end of cholecystectomy the gallbladder was examined intact and the cystic duct ligature was carefully removed. Manual pressure (squeeze) was applied to the fundus of the

gallbladder in an attempt to squeeze the stone out of the cystic duct. Every attempt was made to expel gallstones from the cystic duct. The result of the squeeze test was graded as

- nothing expelled
- debris only expelled from the duct
- debris and stones squeezed out

The results of the "squeeze" test are given below

result	nil	debris	stones
pancreatitis (22)	7	4	11
No pancreatitis (156)	108	31	17

Of all the patients 115/178 (64.6%) had a negative test; 35/178 (19.7%) produced debris; and 28/178 (15.7%) produced gallstones. Analyzing the results as percentages.

	nil	debris	stones
pancreatitis	31.8%	18.2%	50%
No pancreatitis	69.2%	19.9%	10.9%
Significance	P<0.01	N.S.	P<0.01

for overall trend $X^2 = 24.11$, $P < 0.0001$

Thus patients with AGP produced stones more often on squeezing than those without pancreatic disease. This may be as a result of patients with AGP having both;

(i) smaller gallbladder stones

(ii) wider cystic ducts

11. Number of choledochal stones (664 patients)

More patients with pancreatitis had choledocholithiasis than those without pancreatic disease.

	pancreatitis	No pancreatitis	Total
stones in common bile duct	13 (25%)	81 (13%)	94
No stones	39 (75%)	531 (87%)	570
	52	612	664

$X^2 = 4.5$, $P = 0.033$

There was, however, no difference in the actual number of stones present in the common bile duct.

12. Size of choledochal stones (196 patients)

There was no difference in the size of the choledochal stones in patients with and without AGP.

13. Diameter common bile duct (CBD)

(i) (664 patients) The CBD was first assessed at operation as being

(a) dilated > 10mm.

or (b) non-dilated < 10mm.

AGP was associated with a significantly increased incidence of duct dilation.

	pancreatitis	No pancreatitis	Total
dilated	22	112	134
non-dilated	30	500	530
	52	612	664

$X^2 = 15.7$, $P = 0.0001$

Thus of those patients with AGP 42% had a dilated duct whereas only 18% without pancreatitis had evidence of such dilation.

(ii) The diameter of the common bile duct was more accurately measured in 548 of the 664 patients. This was assessed at operative cholangiography by means of caliper as being the widest diameter to the nearest mm (fig. 46). As the presence or absence of choledochal stones is important in determining duct diameter, these patients were subdivided into

(a) <u>those with choledochal stones</u> (N = 86)

There was no significant difference in duct size between the two groups when stones were present.

	<u>pancreatitis</u>	<u>No pancreatitis</u>
Number of patients	13	73
mean rank	54.8	46.3
mean ± SD diameter (mm)	10.8±1.7	11.0±2.1
median diameter (mm)	11.0	12.0

$Z = 1.294$, $P = 0.196$

(b) <u>those without choledochal stones</u> (N = 462)

The common bile duct diameter was bigger in patients with a history of AGP in the absence of choledochal stones. This result was highly significant.

	<u>pancreatitis</u>	<u>No pancreatitis</u>
Number of patients	39	423
mean rank	347	280.98
mean ± SD diameter (mm)	9.53±3.78	7.57±2.93
median diameter (mm)	10.4	7.8

$Z = 2.96$, $P = 0.0031$

Early operations (within 7 days) in the AGP patients were associated with a significantly wider duct than late operations ($P < 0.01$).

14. Duodenal filling (528 patients)

Duodenal filling was assessed on the operative cholangiogram as occurring on the 1st, 2nd or 3rd film. Those without duodenal filling on any film were scored 0. There was evidence of slightly slower duodenal filling in patients with AGP.

	pancreatitis	No pancreatitis
Number of patients	43	485
Mean rank	239.8	266.7
Median	1.393	2.516

$Z = 1.97$, $P = 0.049$

15. Pancreatic Duct Reflux (540 patients)

Pancreatic duct reflux (PDR) occurred in 104 cholangiograms, an overall incidence of 19.3%.

There was an increased incidence of PDR in patients with pancreatitis (56.8% vs 15.9%).

	pancreatitis	No pancreatitis	Total
Number with PDR	25	79	104
Number without PDR	19	417	436
	44	496	540

$X^2 = 50.76$, $P < 0.0001$

Examples of PDR are given in figs. 47A - D.

16. PDR - clinical features

104 patients had PDR on their operative cholangiogram. Of these 25 had a past history of pancreatitis and 79 had no such history. These 104 patients were analysed for various features to determine whether differences were present between those patients with and without AGP.

(a) sex.

	pancreatitis	No pancreatitis
Males	12 (48%)	22 (27%)
Females	13 (52%)	57 (73%)

$X^2 = 3.74$, $P > 0.05$ Not significant.

(b) age.

	pancreatitis	No pancreatitis
Median (range)	58 (24-76)	46.2 (18-78)
Mean ± SD	54.5±16.11	46.5±16.5

$t = 2.15$, $P < 0.02$

Patients with pancreatitis were significantly older.

(c) jaundice.

	pancreatitis	No pancreatitis
jaundice	4 (16%)	18 (23%)
No jaundice	21 (84%)	61 (77%)

$X^2 = 0.21$, not significant.

(d) common bile duct stones.

	pancreatitis	No pancreatitis
Stones	10 (40%)	7 (9%)
No stones	15 (60%)	72 (91%)

$X^2 = 13.46$, $P < 0.001$

There was a highly significant difference between the two groups of patients with respect to the presence of choledochal stones. Patients with a history of pancreatitis and PDR had stones present in the common bile duct far more frequently than those without pancreatic disease.

17. Size of the pancreatic duct (43 patients) (fig. 42).

This was measured on the cholangiogram by caliper to the nearest mm in its widest portion. The pancreatic duct was significantly larger in those patients who had PDR associated with pancreatitis.

	pancreatitis	No pancreatitis
Number of patients	18	25
Mean ± SD diameter (mm)	3.8±0.9	2.13±0.8
Median (range) diameter (mm)	3.5 (2-6)	2.0 (1-4)

$U = 63.5$, $P = 0.000017$

18. Angle of reflux (43 patients) (fig. 42).

The angle between the distal common bile duct and pancreatic duct was measured by protractor to the nearest five degrees on the operative cholangiogram. The angle of reflux was greater in those patients with pancreatitis.

	pancreatitis	No pancreatitis
Number of Patients	18	25
Mean ± SD angle (°)	31.4±10	22.3±9
Median (range) angle (°)	31 (15-52)	20 (8-50)

$t = 3.07$, $P < 0.01$

19. Length of reflux (104 patients) (fig. 42).

The maximum extent of reflux down the pancreatic duct on the cholangiogram was measured to the nearest mm.

	pancreatitis	No pancreatitis
Number of Patients	25	79
Mean ± SD extent of reflux (mm)	30.96±19.9	38.63±30.86
Median (range) extent (mm)	27.25 (5-90)	27.25 (7-155)

t = 1.44, not significant.

The length of reflux was similar in the two groups of patients.

20. Length of common channel (43 patients) (fig. 42).

The length of the common channel was measured by caliper to the nearest mm on those operative cholangiograms where PDR occurred. There was a wide variation in the length (2-44 mm). Patients with pancreatitis had a longer common channel than those without pancreatic disease.

	pancreatitis	No pancreatitis
Number of Patients	18	25
Median (range) length of common channel (mm)	8 (4-16)	4 (2-44)

U = 98.5, P = 0.0015

21. Long term follow up (664 patients).

Of the 52 AGP patients none developed recurrent gallstones or another attack of acute pancreatitis Two developed signs

of pancreatic insufficiency but in both of these alcohol was thought to be a contributing factor (history from patient and wife)

None of the 612 control patients developed acute pancreatitis despite seven returning with recurrent choledochal stones.

The significant results between the two groups of patients are summarized in table 30.

TABLE 30 Significant differences between patients with AGP and controls
(mean ± SD; median & range)

Factor	AGP	No pancreatitis (controls)	Significance (P value)
Male	48%	25%	
Female	52%	75%	0.004[a]
number of gallbladder calculi	21.46±13.11	16.69±4.31	0.0464[b]
size of smallest gallbladder calculi (mm)	2.68±3.43	4.64±4.10	0.0006[b]
diameter of cystic duct (mm)	4.94±2.29	3.68±2.04	0.0001[b]
diameter of CBD : no stones (mm)	9.53±3.78	7.57±2.93	0.0031[b]
Pancreatic Duct Reflux : pancreatic duct diameter (mm)	56.8% 3.8 ± 0.9	15.9% 2.13±0.8	<0.0001[a] <0.0001[b]
: length of common channel (mm)	8(4-16)	4(2-44)	0.0015[b]
: angle of reflux (°)	31.4 ± 10	22.3 ± 9	<0.01[b]
Squeeze test stones	52.4%	10.9%	<0.0001[a]

a Chi - Square

b Mann - Whitney u test

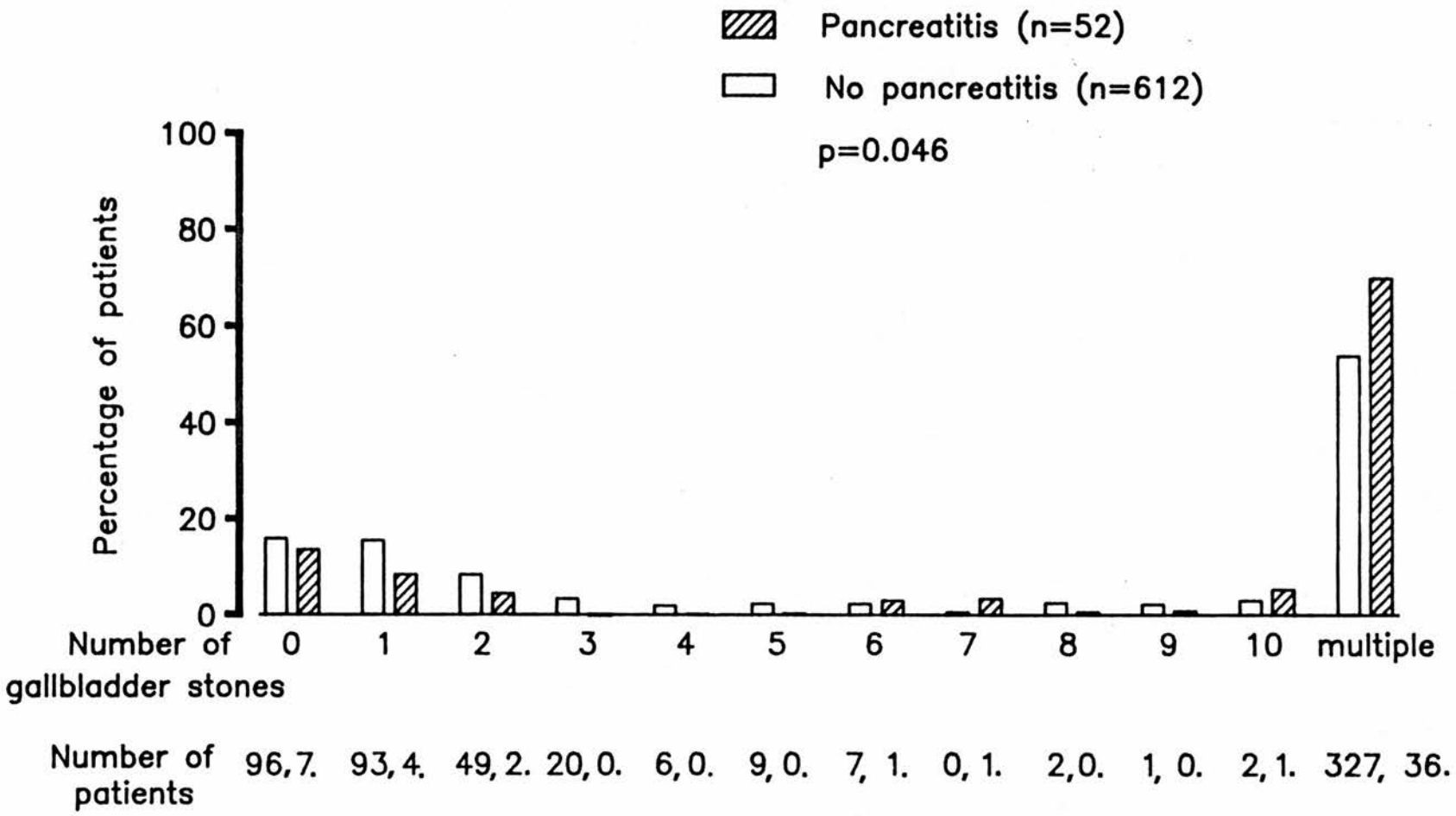


Fig. 43 Number of gallbladder calculi.

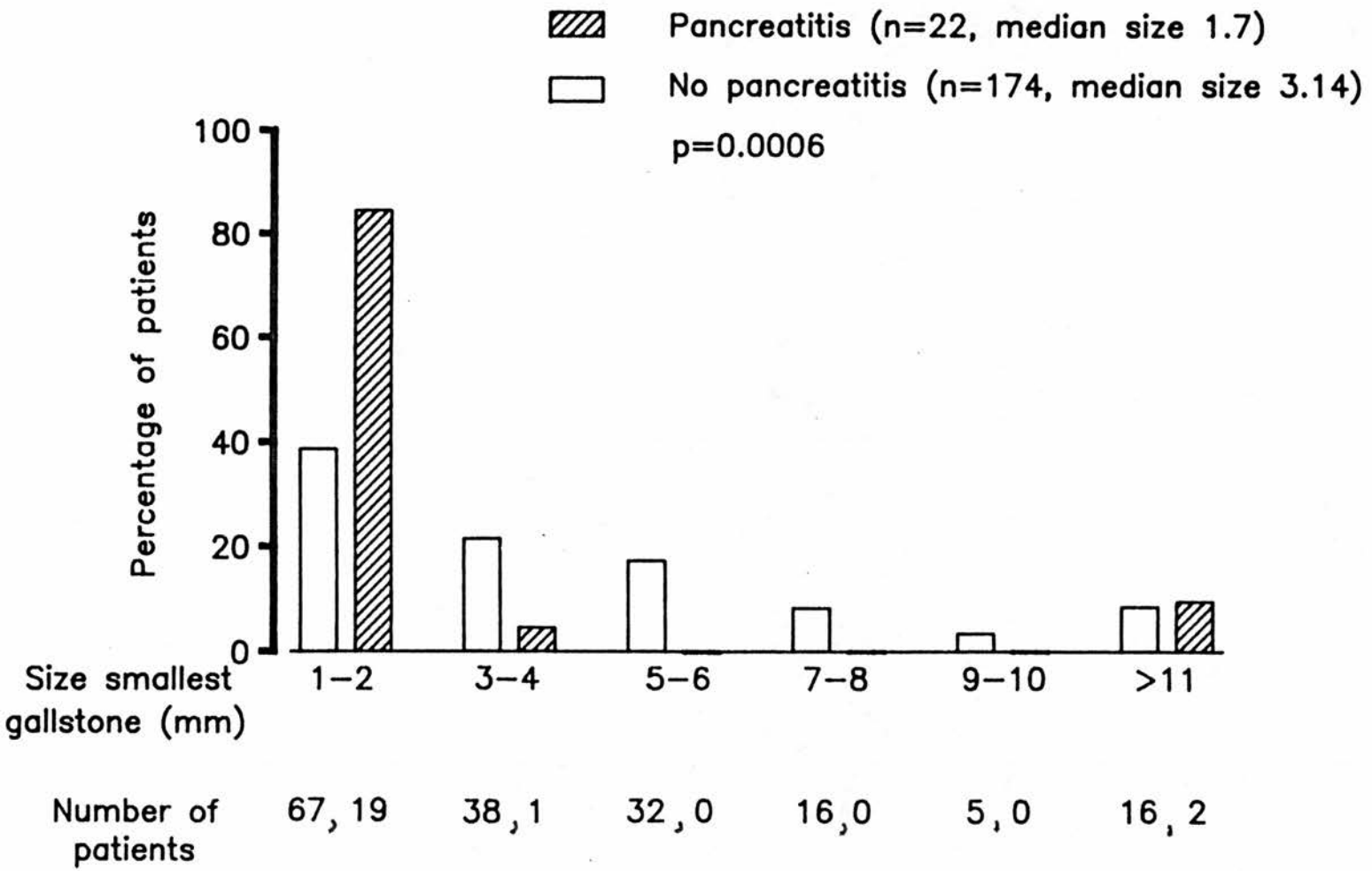


Fig. 44 Size of smallest gallbladder calculi.

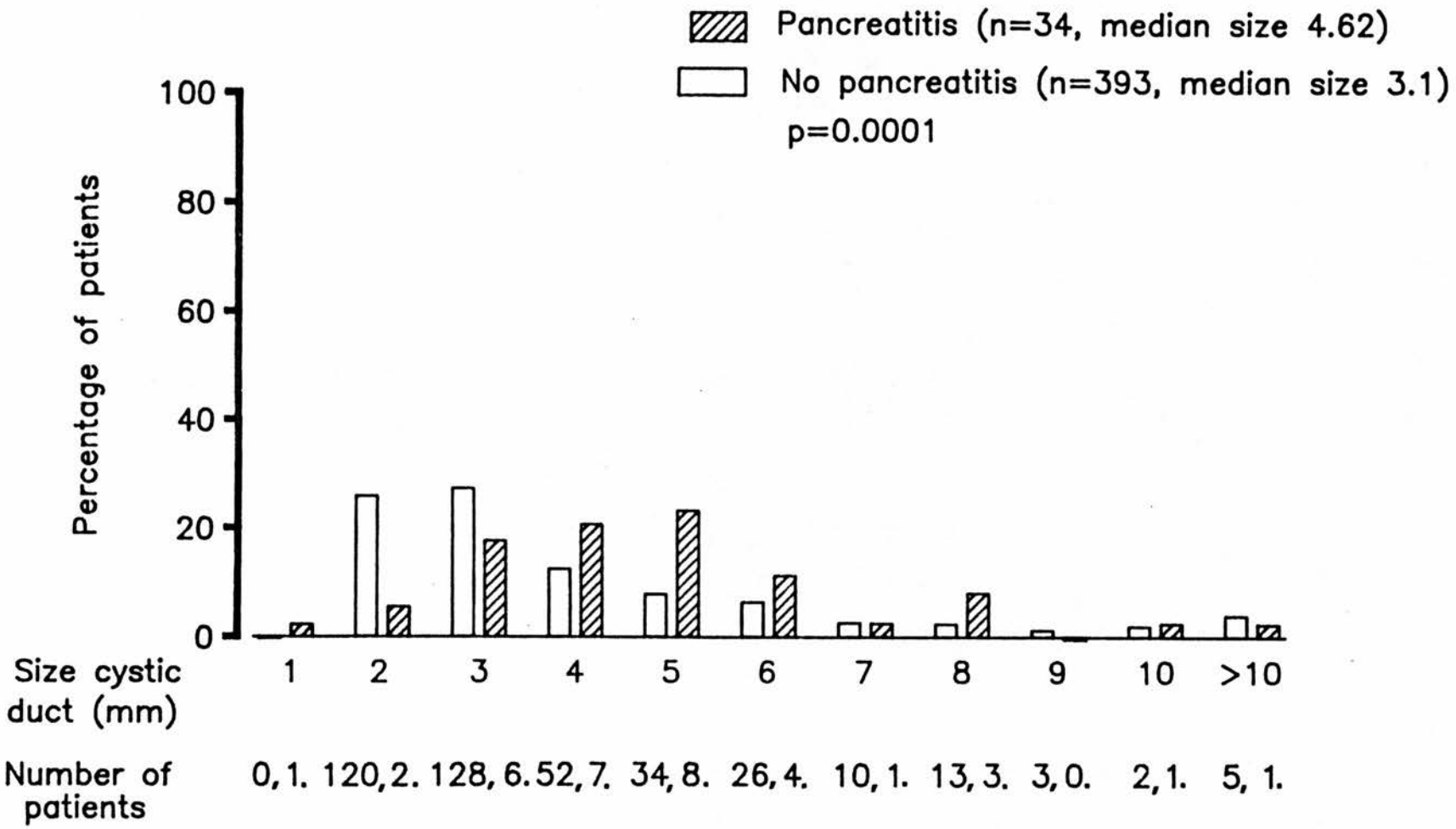


Fig. 45 Size of cystic duct.

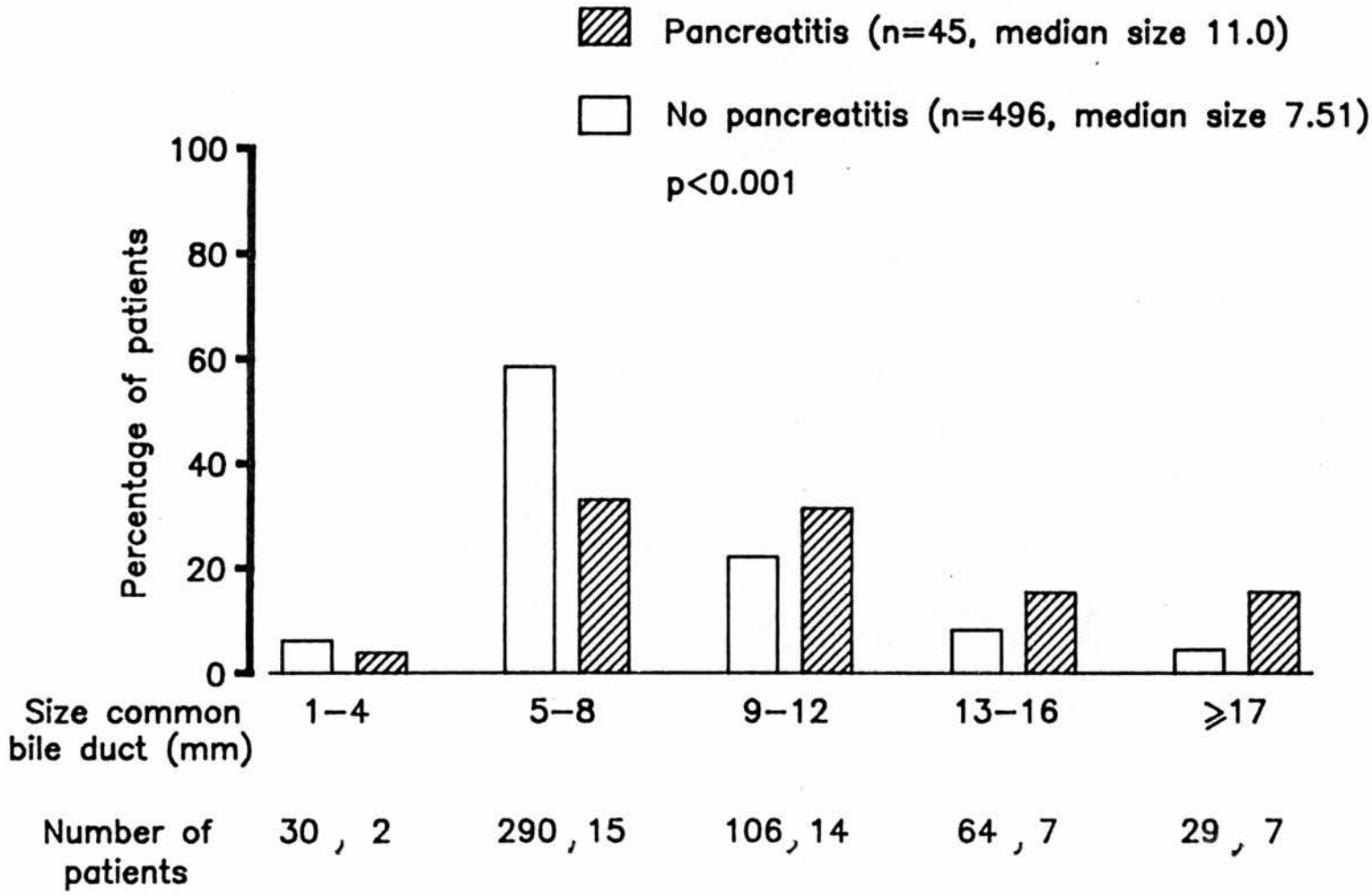


Fig. 46 Size of common bile duct.

Fig. 47A Operative cholangiogram: Pancreatic duct reflux down whole pancreatic duct.

Note long common channel (44 mm).
angle of reflux 32°.

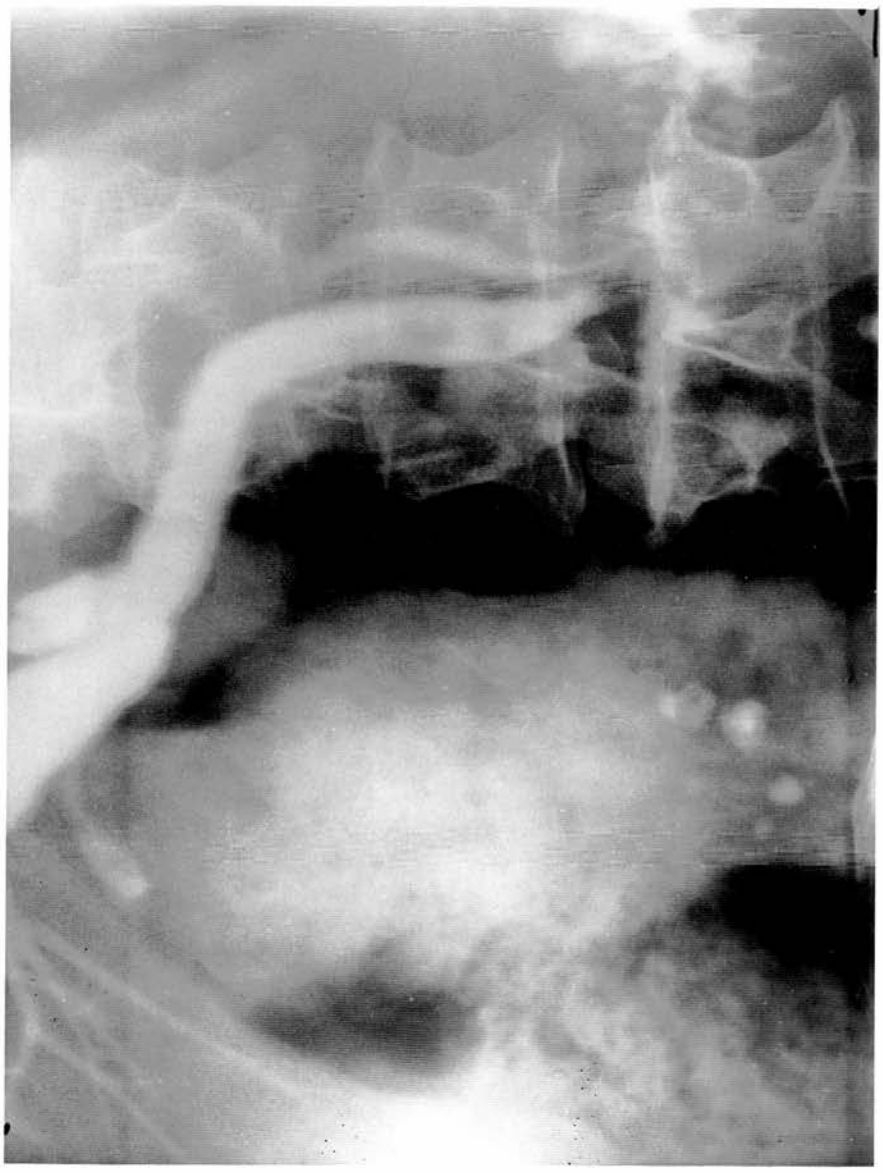


Fig. 47B Operative cholangiogram. Pancreatic-duct reflux.

Note stones in common bile duct, ampulla in third part of duodenum.

Fig. 47C Operative cholangiogram showing Pancreatic - Duct Reflux.

Note loop in pancreatic duct.

Fig. 47D Operative Cholangiogram. Pancreatic-Duct Reflux.

Note duodenal diverticulum.

Discussion

This prospective study has collected a large data base on a group of patients with cholelithiasis. Of these 664 patients, 52 (7.8%) had suffered an attack of acute gallstone pancreatitis, an incidence slightly higher than that quoted in the literature (Braganza 1983b, Carter 1983, Ranson 1979). Males with gallstones were at greater risk than females of developing AGP as in this study 48% of patients with AGP were male compared with only 24% of those without a history of pancreatitis. The reason for this observation is unclear although it has been previously noted (Taylor 1981). There was no significant difference between the two groups of patients (AGP present or not) as regards age and a history of jaundice.

It is well recognized that the timing of biliary surgery, following an attack of pancreatitis, greatly influences the operative findings as the earlier one operates the more frequently are stones found in the common bile duct and at the ampulla of Vater. In this study the timing of surgery was compared with the common bile duct and ampullary findings. Only three patients underwent urgent early surgery (< 2 days) and of these two had choledochal stones with stones also impacted at the ampulla. These figures are similar to the findings of Acosta (1980) and Stone (1981), who demonstrated impacted stones in 75% of patients undergoing surgery at less than two days.

The more delayed is surgery after an attack of pancreatitis the less often are calculi found in the common bile duct and at the ampulla. In this study there was a steady reduction in the number of stones found in the common bile duct and at the ampulla when compared to delay in operation. In patients undergoing operation at 8-21 days only 23% had choledochal

stones and one an ampullary stone. Operating after three weeks, as is standard practice in many hospitals, choledochal stones were found in only 12% of patients and ampullary stones in 4%. These figures further evidence the concept of gallstone migration and confirm the observations of Acosta (1980), Kelly (1980), Stone (1981) and Osborne (1981).

In this study there was an increased mortality rate in patients undergoing surgery after an attack of pancreatitis. The five times increased mortality rate is explained by the fact that several emergency operations were performed on patients with acute haemorrhagic pancreatitis, with the attendent increased mortality (Ranson 1981). Indeed the mortality rate in patients undergoing non emergency surgery was similar in the two groups. The only post-operative complication that was more common was that of wound infection. It is now well recognized that wound infection following biliary surgery is often a result of contamination with biliary organisms (Keighley 1983). This suggests that patients with gallstone pancreatitis have a higher incidence of infection in the biliary tree and relates closely to my findings in the first part of this thesis. Further study of biliary bacteriology in patients with pancreatitis is warranted. It is noteworthy that one patient in the AGP group developed fatal pancreatitis following surgery, emphasizing the importance of careful biliary operations in these patients. Only one control patient developed mild post-operative pancreatitis.

Patients in this study with AGP tended to have more gallstones in their gallbladders than patients without pancreatic disease. This result may be more significant than the statistics show as multiple stones were arbitrarily coded as 30 and many patients with pancreatitis had hundreds

of small stones. These results are in agreement with McMahon and Shefta (1980) who demonstrated that 78% of patients with pancreatitis had more than five gallbladder stones whereas only 61% without pancreatic disease had such numbers. The present study gives equivalent figures of 76% and 56% respectively.

It is now generally accepted that small gallstones migrating down the biliary tree are important in initiating gallstone pancreatitis and both Acosta (1974, 1980) and Kelly (1976, 1980) have demonstrated that these stones are usually less than 5 mm in diameter. McMahon (1980) further showed that small irregular stones were responsible and these stones were lighter in those patients with pancreatitis. In this study the sizes of the smallest and largest gallbladder stones were carefully measured. Whereas there was no difference in the relative sizes of the largest gallstones between the two groups of patients there was a highly significant ($P = 0.0006$) difference in the sizes of the small stones. Patients with AGP had significantly smaller stones (1.7 mm vs 3.14 mm) suggesting that these small stones were responsible for initiating pancreatic inflammation.

The size of the cystic duct is a critical determinant of gallstone passage, and McMahon (1980) has demonstrated indirectly that the cystic duct in patients with AGP is bigger than in those without pancreatic disease. This study measured the actual diameter of the cystic duct at operation and found a highly significant ($P = 0.0001$) difference between the two groups. The cystic duct was significantly larger (4.62 mm vs 3.1 mm) in those patients with pancreatitis. When the sizes of the small gall-bladder stones and the cystic duct were compared then only stones in

patients with AGP appeared able to pass through the cystic duct, i.e.

	pancreatitis	No pancreatitis
mean size small gallstones (mm)	1.7	3.14
mean size cystic duct (mm)	4.62	3.1
	passage	no passage

The size of the cystic duct is on average two to three times greater than that of the smallest stone in patients with pancreatitis. In contrast the similar sizes in control patients hinders stone passage. Although it is uncertain whether the dilated cystic duct is a primary or secondary phenomenon, these measurements do confirm that small gallstones can easily migrate through the cystic duct in patients with pancreatitis.

An attempt to examine stone passage through the cystic duct led us to develop the "squeeze" test. This test applies manual high pressure to the gall-bladder and examines extrusion of stones and debris from the cystic duct. In patients without pancreatic disease, stones passed in only 11% of patients, debris in 20% and nothing in 69%. In contrast, in patients with pancreatitis stones passed in 50%, debris in 18% and nothing in 30%. These results are highly significant and show that in half the patients with pancreatitis stones passed through the cystic duct whereas passage occurred in very few patients without pancreatic disease. It is of interest that the incidence of debris passage remained constant between the two groups suggesting that stones and not debris are responsible for initiating pancreatic inflammation.

In this study more patients with pancreatitis had choledocholithiasis. However, this result may be fallacious as we have previously shown the

time of operation to be a critical determinant of biliary pathology and there has been a recent move to earlier surgery. There was no difference in the size of choledochal stones between the two groups of patients whereas the gallbladder stones were previously shown to be significantly smaller in patients with AGP. In the context of gallstone migration it may be that small stones have migrated into the gut whereas the larger stones remain trapped in the common bile duct.

The size of the common bile duct is used both radiographically and at operation to determine the presence of biliary obstruction. In this study the common bile duct was more often dilated in patients with pancreatitis implying a degree of obstruction to the outflow of bile. Closer examination of the common bile duct diameter with respect to the presence of choledochal stones showed that in patients with choledochal stones there was no difference in common bile duct diameter between those with or without pancreatitis. In contrast when choledochal stones were absent there was a highly significant ($P = 0.0001$) difference between the groups. Patients with AGP and no choledochal stones had a much wider common bile duct than those patients without pancreatitis. Indeed the presence or absence of choledochal stones had much less effect on the common bile duct diamter in patients with AGP than in patients without pancreatic disease. These results are in close agreement with those of Osborne and colleagues (1983) and suggest that stones had recently passed down the biliary tree causing temporary obstruction of the common bile duct. The findings of a wider duct in early operations further evidences this theory.

Duodenal filling on operative cholangiography is dependent on free flow

through the ampulla of Vater. In this study the slightly delayed filling in patients with AGP implied partial obstruction to free flow. Whether this was due to gallstones or oedema at the ampulla of Vater is uncertain, although this observation does clearly add further weight to the concept of temporary obstruction of the biliary tree.

Pancreatic duct reflux (PDR) is often observed during operative cholangiography although its relationship to previous pancreatitis was not recognized until recently. It is now well established that patients with previous pancreatitis have a much higher incidence of PDR than those without pancreatic disease (Taylor 1980, Kelly 1976) and this reflux is associated with an elevation in serum amylase activity (Thomas 1983, Howell 1950). PDR is independent of choledochal stones although it becomes both more frequent and striking when stones are impacted at the ampulla. The largest reported study is that of Taylor and Rimmer (1980) who found PDR to occur in 52% of patients with AGP in contrast to only 17% when no pancreatic disease was present. This report gives results which are very similar to those of Taylor and Rimmer (1980). In this study PDR occurred in 19% of all cholangiograms, with the increased incidence of PDR in patients with AGP confirmed as PDR was observed in 57% of patients with pancreatitis against only 16% of those without.

PDR in itself is a relatively crude assessment of reflux as it may occur to a small degree or down the entire pancreatic duct. Indeed why does PDR occur in some patients without pancreatic disease? This study has taken cognisance of these points and has therefore measured several features of PDR. In those patients with PDR there was no difference in sex or the

presence of jaundice although patients with pancreatitis and PDR tended to be older than those without pancreatitis ond PDR. PDR was much more closely associated with the presence of choledochal stones in patients with pancreatitis than in those without. It may be that in those patients without pancreatitis reflux occurs as a result of ampullary anatomy whereas in those with pancreatitis such reflux is more dependent on stone passage through the biliary tree.

The diameter of the pancreatic duct was carefully assessed on operative cholangiograms where PDR occurred. Patients who had a previous attack of pancreatitis had a significantly more dilated pancreatic duct than those without pancreatic disease and this result was extremely significant ($P = 0.000017$). Although it is debateable whether dilation is primary or secondary to the pancreatic inflammation, there is no doubt that a dilated pancreatic duct will mechanically faciliate reflux by reducing the natural resistance to such reflux. It is of interest that Csendes (1979) has demonstrated that the mere presence of gallstones is associated with a greatly dilated pancreatic duct. Another factor not previously described, which may be important in determining ease of PDR is the angle at which the two ducts joined. This was carefully measured on the cholangiogram films and demonstrated a greater angle of reflux in those patients with pancreatitis. The angle of reflux appears to be another factor which mechanically facilitates pancreatic duct reflux.

The length of PDR observed was variable (5-155 mm) and appeared unrelated to the presence or absence of pancreatic disease. In contrast the common channel was significantly ($P = 0.0015$) longer in patients with pancreatitis implying that a stone present at the ampulla would allow biliary reflux

down the pancreatic duct. This confirms earlier reports by Kelly (1976, 1982).

Pancreatic duct reflux is more common in patients with pancreatitis. It appears to occur more easily in these patients as a result of reduced mechanical obstruction to such reflux (wider pancreatic duct and increased angle of reflux). Furthermore the longer common channel in patients with PDR and pancreatitis suggests that stone passage through the ampulla is associated with such reflux.

Several interesting results have derived from this study and there is no doubt in the author's mind that the observed features of the biliary tree in patients with AGP are consistent with the concept of gallstone migration. Why some patients have pancreatic duct reflux and do not develop pancreatitis is unknown but it is tempting to speculate that the nature of refluxed bile is important as was earlier shown in parts I and II of this thesis.

Finally, the efficacy of gallstone eradication in preventing further pancreatic disease was confirmed. During the follow up period no patients developed either further gallstones or another attack of pancreatitis implying that gallstones are essential mechanical initiators of AGP. Two patients developed chronic pancreatitis but in both of these alcoholism was thought to be a contributing factor.

The conclusions to be drawn from this study are:

(1) 8% of patients with gallstones develop acute pancreatitis.

(2) Males with gallstones are at greater risk of developing

pancreatitis.

(3) The timing of surgery is a critical determinant of biliary pathology. The earlier one operates after the onset of pancreatic inflammation the more stones are found at the ampulla and in the common bile duct.

(4) Wound infection was more common in patients with pancreatitis implying a role for infected bile.

(5) The gallbladder stones in patients with pancreatitis were more numerous and smaller than in those without pancreatic disease.

(6) The cystic duct diameter was much bigger in patients with pancreatitis .

(7) Stone passage through the cystic duct was much easier in patients with pancreatitis.

(8) The common bile duct was wider in the absence of stones in those patients with previous pancreatitis, suggesting previous temporary biliary obstruction.

(9) The common bile duct stones were of similar size in the two groups of patients implying that small stones had migrated into the gut.

(10) Pancreatic duct reflux is much commoner in patients with previous pancreatitis. When it does occur there is a wider pancreatic duct, a greater angle of reflux and a longer common channel in those patients with pancreatitis.

(11) Appropriate biliary surgery with eradication of stones will prevent further attacks of gallstone pancreatitis.

CHAPTER XIV

Final Discussion

Acute gallstone pancreatitis (AGP) is a common disease with a mortality rate of 10-15%. Although this condition has been the subject of intense research there is still considerable controversy over the initiation of pancreatic inflammation. This thesis has approached the problem in three ways:

(1) Induction of acute pancreatitis in an animal model and study of the factors possibly involved.

(2) Investigation of pancreatic duct integrity by means of studying duct permeability and ultrastructure.

(3) A study of the biliary tract in patients with acute gallstone pancreatitis.

part I

Reviewing the literature showed that a return to simple first principles was necessary for the study of AGP. The initial part of this research was concerned with the development of an animal preparation for inducing pancreatitis. A model using transduodenal cannulation of the rat bile-pancreatic duct was studied which gave reproducible and well-defined pancreatic damage. It was determined that 50 µl was the optimum volume for infusion thus bringing into question the results of previous experiments. The pressure of infusion was important in producing pancreatic damage as saline alone produced oedema which increased with both time and pressure of infusion. The duct system was intact at low pressures, at moderate pressures extravasation occurred through intercellular clefts, and at high pressures the duct ruptured. These results suggest that it is vital

to control volume, pressure and time when infusing various noxious substances into the pancreas. Having determined the optimum physical conditions for infusion the next study concerned the effects of bile alone on the pancreas. Several types of human bile were studied; sterile, "pancreatitic", infected and filtered. Sterile bile was only toxic when infused at high pressures and even then only moderate pancreatitis occurred. "Pancreatitic" bile was significantly more toxic than sterile bile in all specimens examined, suggesting that bile in patients with AGP has a different chemical makeup which renders it more damaging to the pancreas. Infected bile consistently produced acute haemorrhagic pancreatitis. This was not because of the bacteria themselves but rather by secondary alterations in bile induced by bacteria. Thus even at low pressures "pancreatitic" and infected bile produced severe pancreatic inflammation.

The toxicity of bile or duodenal juice may be due to several substances and of these bile salts, trypsin, enterokinase, phospholipase A_2 and lysolecithin are probably the most important. Their relative importance in producing pancreatic inflammation at presumptive pathophysiological concentrations was evaluated. Whereas enterokinase or trypsin alone produced pancreatic oedema and bile salts were responsible for mild inflammation, a combination of these compounds consistently produced acute haemorrhagic pancreatitis. These results suggest that bile salts, enterokinase and trypsin may have a role in the initiation of pancreatic damage. Active phospholipase A_2 and lysolecithin were also toxic to the pancreas producing acinar necrosis and acute inflammation and these compounds can also be considered as important factors in producing pancreatic damage.

Part I of this study identified several factors that might be responsible for the initiation of AGP. The most serious damage occurred as a result of infusion with

- infected bile
- "pancreatitic" bile
- bile salt/enterokinase/trypsin mixture
- lysolecithin/phospholipase A_2

part II

Damage to the pancreatic duct system appears to be the initial insult in AGP and this damage may be resultant on bile or duodenal reflux into the pancreatic ducts. Study of pancreatic duct integrity could therefore yield important information concerning the initiation of pancreatic inflammation. Reber and colleagues (1979) have described a "pancreatic duct mucosal barrier", (i.e. the property of the duct wall that prevents free diffusion) following experiments with the feline main pancreatic duct. As the cost of cats is now prohibitive, we evaluated the possibility of using the rat bile-pancreatic duct to study duct integrity. The rat BPD was structurally very similar to the pancreatic duct and was lined by the same specialized epithelium. The permeability characteristics of the rat BPD determined it to be a suitable model for studying duct integrity, or the "pancreatic duct mucosal barrier". Stability of the "barrier" was measured in three ways: (i) anionic flux of Cl^- and HCO_3^-, (ii) transductal potential difference and (iii) mucosal ultrastructure. This allowed a full assessment of duct integrity and was superior to the earlier methods of Reber (1979) and Simpson (1983).

Substances which were studied in part I were further evaluated for their

direct effect on the duct wall. Pressure alone produced widened intercellular spaces but no change in "barrier" integrity. Sterile bile produced moderate damage to the duct which was accentuated by high pressure. The changes observed were reversible and the overall duct structure was maintained. As in part I "pancreatitic" bile was more toxic than sterile bile and there was evidence of epithelial necrosis and disruption. Infected bile produced the most severe damage with the duct being completely disrupted. These results are in keeping with the earlier experiments in part I and suggest (i) bile from patients with pancreatitis has special noxious properties and (ii) infected bile is extremely destructive to the duct wall, possibly resultant on bacterially induced changes in bile.

Bile contains two powerful natural detergents; bile salts and lysolecithin. Bile salts and in particular glycodeoxycholic acid have long been recognized as being unhealthy to cell membranes. This study assessed the effect of bile salts on the duct and found them to be extremely damaging. Low concentrations produced reversible changes whereas high concentrations of bile salt were associated with severe epithelial disruption. The other naturally occurring powerful detergent is lysolecithin produced by phospholipase action. Active PLA_2 and lysolecithin were extremely destructive to the duct, and the changes identified appeared to be irreversible with evidence of cell death and shedding into the lumen.

Why are pure solutions of bile salts and lysolecithin much more destructive than whole bile which contains equal concentrations of these compounds? This question stimulated an investigation of possible modifying agents in bile. Prostaglandins, which are present in small amounts in bile, were

partially protective against damage induced by bile salts and PLA_2/lysolecithin. More importantly lecithin, an obligatory constituent of whole bile, was almost completely effective in preventing detergent induced damage. It may be that lecithin combines with bile salts and lysolecithin and so reduces their surface active properties. Of interest, it has recently been reported that patients with acute cholecystitis have reduced levels of lecithin in their bile (Heuman 1980) which appears to make the bile more toxic to the gall bladder. It is tempting to postulate that lecithin plays a vital physiological role in protecting biliary and pancreatic epithelium. Reduction in the lecithin:bile salt or lecithin:lysolecithin ratios would therefore result in especially noxious bile.

part III

The third part of this study investigated the biliary tree in patients with gallstones and in particular those with AGP. 664 patients undergoing cholecystectomy for biliary lithiasis were prospectively studied, of whom 52 had gallstone pancreatitis. A comparison of the biliary tree of patients with AGP with that of patients without pancreatic disease highlighted several significant differences;

- more males with gallstones developed AGP than females.
- patients with AGP had an increased number and smaller stones in their gallbladder.
- the diameter of the cystic duct was wider in patients with AGP and stone passage into the biliary tree was facilitated.
- the diameter of the common bile duct in patients with AGP when stones were absent indicating that transient obstruction had occurred. The common duct stones themselves were similar in

size in both groups suggesting that small stones had passed into the duodenum and the large stones were left behind.

- the operative cholangiograms of patients with a history of AGP demonstrated a highly significant increased evidence of pancreatic duct reflux (57% vs 16%).
- complete removal of gallstones obviated further attacks of pancreatitis.

A further study was made on 104 patients who had evidence of pancreatic duct reflux. In patients with pancreatitis and PDR there was a significantly

(i) longer common channel.

(ii) wider pancreatic duct.

(iii) greater angle of reflux

These factors may facilitate bile or duodenal reflux following gallstone migration through the ampulla of vater.

These patient studies are important in that they:

(a) support the concept of gallstone migration.

(b) suggest that passage of small stones down the biliary tree is responsible for initiating pancreatic inflammation.

(c) show that pancreatic duct reflux is a vital part of the disease process.

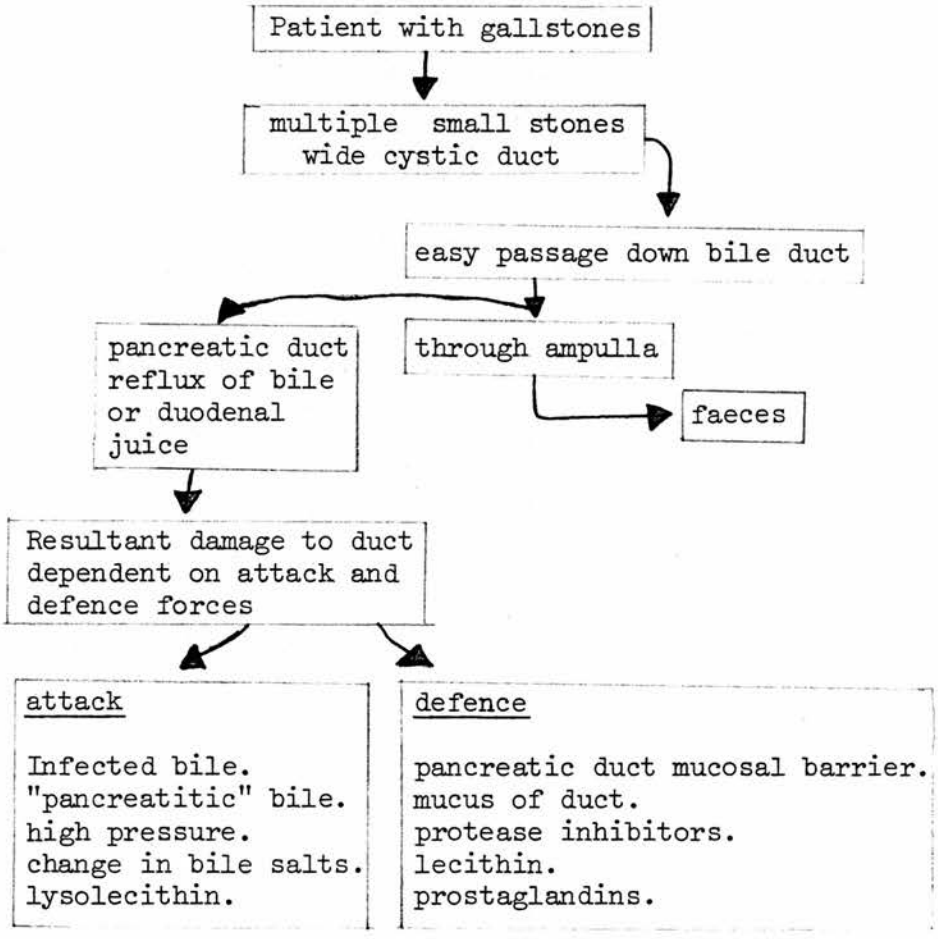
and (d) demonstrate that the biliary tree and gallstones are significantly different in patients with AGP.

Having identified factors which are associated with AGP we might soon be able to predict those patients with gallstones who are at greatest risk of developing acute pancreatitis and hence offer them early surgery.

It is evident, however, from these studies that gallstone migration is only the mechanical initiator. As pancreatic reflux can occur in some patients without troublesome sequelae it appears that the nature of the refluxed material is important. This concept corresponds closely to the results of parts I and II.

Hypothesis

This research has enabled me to hypothesize on factors which might be relevant in initiating acute gallstone pancreatitis.



Im patients who develop AGP I believe that

(i) the anatomy of the biliary tree and nature of the

gallstones are such as to mechanically produce pancreatic duct reflux

and (ii) following reflux there is increased attack and reduced defence forces in the pancreatic duct.

This work has opened up exciting new prospects for studying acute gallstone pancreatitis. A full evaluation of biliary and duodenal constituents in such patients might well be invaluable in determining the pathogenesis of this dangerous condition.

REFERENCES

1. Acosta, J.M., Ledesma, C.L.
Gallstone migration as a cause of acute pancreatitis.
New Eng. J. Med. 1974; 290: 484-6.

2. Acosta, J.M., Rossi, R., Galli, O., Pellegrini, C., Skinner, D.B.
Early surgery for acute gallstone pancreatitis: Evaluation of a systematic approach.
Surgery 1978; 83: 367-70.

3. Acosta, J.M., Pellegrini, C.A., Skinner, D.B.
Etiology and pathogenesis of acute biliary pancreatitis.
Surgery 1980; 88: 118-23.

4. Adler, G., Rohr, G., Kern, H.F.
Alteration of membrane fusion as a cause of acute pancreatitis in the rat.
Dig. Dis. Sci. 1982; 27: 993-1002.

5. Aho, H.J., Nevalainen, T.J.
Experimental pancreatitis in the rat. Sodium taurocholate - induced acute haemorrhagic pancreatitis.
Scand. J. Gastroent. 1980a; 15: 411-16.

6. Aho, H.J., Nevalainen, T.J.
Experimental pancreatitis in the rat. Ultrastructure of sodium taurocholate - induced pancreatic lesions.
Scand. J. Gastroenterol. 1980b; 15: 417-24.

7. Aho, H.J., Nevalainen, T.J., Lindberg, R.L.P., Aho, A.J.
Experimental pancreatitis in the rat. The role of phospholipase A_2 in sodium taurocholate - induced acute haemorrhagic pancreatitis.
Scand. J. Gastroenterol. 1980c; 15: 1027-31.

8. Aho, H.J., Nevalainen, T.J.
Experimental pancreatitis in the rat. Light and electron microscopical observations on early pancreatic lesions induced by intraductal injection of trypsin, phospholipase A_2, lysolecithin and non-ionic detergent.
Virchows Arch (Cell Pathol.) 1982; 40: 347-56.

9. Aho. H.J., Nevalainen, T.J., Aho, A.J.
Experimental pancreatitis in the rat. Development of pancreatic necrosis, ischaemia and oedema after intraductal sodium taurocholate injection.
Eur. Surg. Res. 1983; 15: 28-36.

10. Allen, B., Garner, A.
Mucus and bicarbonate secretion in the stomach and their possible role in mucosal protection.
Gut 1980; 21: 249-62.

11. Ammon, H.V.
Effect of taurine conjugated bile salts with and without lecithin on water and electrolyte transport in the canine gallbladder in vivo.
Gastroenterology 1979; 76: 778-83.

12. Anacker, H., Weiss, H.D., Kramma, B.
Endoscopic retrograde pancreaticocholangiography.
Berlin: Springer-Verlag, 1977.

13. Anderson, M.C., Van Hagen, F., Method, H.L., Mehn, W.H.
An evaluation of the use of bile and trypsin in the production of experimental pancreatitis.
Surg. Gynecol. Obstet. 1958; 107: 693.

14. Anderson, M.C., Hagstrom, W.J.
A comparison of pancreatic and biliary pressures recorded simultaneously in man.
Can. J. Surg. 1962; 6: 461-70.

15. Anderson, M.C., Lewis, M.B.
Low molecular weight dextran therapy in experimental pancreatitis.
JAMA 1965; 192: 398-401.

16. Anderson, M.C., Schiller, W.R.
Microcirculatory dynamics in the normal and inflamed pancreas.
Am. J. Surg. 1968; 115: 118-27.

17. Anderson, M.C., Needleman, S.B., Gramatica, L., Toranto, I.R., Briggs, D.R.
Further inquiry into the pathogenesis of acute pancreatitis. Role of pancreatic enzymes.
Arch. Surg. 1969; 99: 185-92.

18. Anderson, M.C., Schiller, W.R.
Acute pancreatitis.
Surgical Annual 1973; 5: 335-54.

19. Anderson, R.J.L., Jeffrey, I.J.M., Kay, P., Braganza, J.M.
Lipid peroxidation products and experimental pancreatitis.
Presentation to Pancreatic Society of Great Britain, Manchester, 1983.

20. Arnesjö, B., Barrowman, J.A., Borgstrom, B.
The zymogen of phospholipase A_2 in rat pancreatic juice.
Acta Chem. Scand. 1967; 21: 2897-99.

21. Arnesjö, B.
The formation of lysolecithin in rat pancreas after experimentally induced bile salt pancreatitis.
Acta. Chir. Scand. 1971; 137: 351-4.

22 Austin, J.L., Sieckman, G.
Effect of pancreatic duct pressure on pancreatic duct permeability to macromolecules.
Gastroenterology 1984; 86: 1017 (Abst).

23. Baba, N., Inoue, K., Chang, L.W., Rayford, P.L.
Effect of obstructive jaundice on the pancreas of rats.
Gastroenterology 1983a; 28: 1095 (Abst).

24. Baba, N., Chowdury, K., Inoue, K., Rayford, P.L.
Effect of ileal and caecal resection on pancreatic growth and weight of GI tract organs in rats.
Dig. Dis. Sci. 1983b; 28: 925 (Abst).

25. Baggenstoss, A.H.
Pathology of pancreatitis.
In: Pancreatitis. (Ed) Gambill E.E., St Louis, C.V. Mosby, 1973.

26. Banks, P.A.
Acute pancreatitis.
Gastroenterology, 1971; 61: 382-97.

27. Banks, P.A.
Pancreatitis.
New York. Plenum Book Co., 1979.

28. Bar-Meir, S., Geenen, J.E., Hogan, W.J., Dodds, W.J., Stewart, E.T., Arndorfer, R.C.
Biliary and pancreatic duct pressures measured by ERCP manometry in patients with suspected papillary stenosis.
Dig. Dis. Sci. 1979; 24: 209-13.

29. Bawnik, J.B., Orda, R., Witnitzer, T.
Acute necrotizing pancreatitis. An experimental model.
Am. J. Dig. Dis. 1974; 19: 1143-47.

30. Beck, I.T., Pinter, E.J., Solymar, J., McKenna, R.D., Ritchie, A.C.
The role of pancreatic enzymes in the pathogenesis of acute pancreatitis. II. The fate of pancreatic proteolytic enzymes in the course of acute pancreatitis.
Gastroenterology 1962; 43: 60-70.

31. Beck, I.T., Kahn, D.S., Solymar, J., McKenna, R.D., Zylberzac, B.
The role of pancreatic enzymes in the pathogenesis of acute pancreatitis III. Comparison of the pathological and biochemical changes in the canine pancreas to intraductal injection with bile and trypsin.
Gastroenterology 1964; 46: 531-5.

32. Beck, I.T.
Histolopathology of acute pancreatitis.
In: The exocrine pancreas. Eds. Beck, I.T., Sinclair, D.G.
London, Churchill 1971.

33. Becker, J.M., Ti, T.K., Moody, F.G., McGreevy, J.M., Nelson, J.A.
A model of biliary pancreatic reflux.
Surgery. 1979; 85: 147-53.

34. Becker, J.M., Moody, F.G., Zionmeister, A.R.
Effect of gastrointestinal hormones on the biliary sphincter of the opossum.
Gastroenterology 1982; 82: 1300-7.

35. Behar, J., Biancani, P.
Effect of cholecystokinin on the feline sphincter of oddi and gallbladder. Mechanisms of action.
J. Clin. Invest. 1980; 66: 1231-9.

36. Bell, E.T.
Pancreatitis.
Surgery 1958; 43: 527-37.

37. Berci, G., Johnson, N.
Functional studies of the extrahepatic biliary system in the dog by use of a controlled biliary fistula.
Ann. Surg. 1965; 161: 286-92.

38. Bergh, G.S., Layne, J.A.
A demonstration of the independent contraction of the sphincter of the common bile duct in human subjects.
Am. J. Physiol. 1940; 128: 690-4.

39. Bergh, G.S.
The effect of food upon the sphincter of oddi in human subjects.
Am. J. Dig. Dis. 1942; 9: 40-3.

40. Bernard, C.
Du suc pancréatique et de son rôle dans les phénomènes de la digestion.
C.R. Soc. Biol. (Paris) 1850; 1: 99-115.

41. Berry, A.R.
Acute pancreatitis - respiratory complications and pathogenetic aspects.
ChM Thesis; University of Edinburgh, 1981.

42. Bilbao, K., Dotter, C.T., Lee, T.T.
Complications of endoscopic retrograde cholangiopancreatography.
Gastroenterology 1976; 70: 314-20.

43. Black, R.B., Hole, D., Rhodes, J.
Bile damage to the gastric nucosal barrier. The influence of pH and bile acid concentration.
Gastroenterology 1971; 61: 178-84.

44. Blackwell, G.J., Flower, R.J.
Inhibition of phospholipase.
Br. Med. Bulletin 1983; 39: 260-4.

45. Block, M.A., Wakim, K.G., Baggenstoss, A.H.
Experimental studies concerning factors in the pathogenesis of acute pancreatitis.
Surg. Gynecol. Obstet. 1955; 101: 83-90.

46. Blumenberg, R.M., Powers, S.R.
Acute biliary pancreatitis. An experimental reappraisal of factors governing biliary reflux into the pancreas.
Ann. Surg. 1963; 158: 1058-

47. Blumenthal, H.T., Probstein, J.G.
Pancreatitis.
Springfield: Thomas C.C., 1959.

48. Bockman, D.E., Schiller, W.R., Anderson, M.C.
Route of retrograde flow in the exocrine pancreas during ductal hypertension.
Arch. Surg. 1971; 103: 321-9.

49. Boctor, A.M., Band, P., Grossman, A.
Requirement for an accessory factor for binding of ^{3}H estradiol to protein in the cytosol fraction of rat pancreas.
Proc. Natl. Acad. Sci. USA; 1981: 78: 5648-51.

50. Bolender, R.P.
Stereological analysis of the guinea pig pancreas.
J. Cell. Biol. 1974; 61: 269-87.

51. Boyle, J.M., Neiderhiser, D.H., Dworken, H.J.
Duodenogastric reflux in patients with gastric ulcer disease.
J. Lab. Clin. Med. 1984; 103: 14-21.

52. Bozler, E.
Reflex peristalsis of the intestine.
Am. J. Physiol. 1949; 157: 338-42.

53. Brackett, K.A., Crocket, A., Joffe, S.N.
Ultrastructure of early development of acute pancreatitis in the rat.
Dig. Dis. Sci. 1983; 28: 74-84.

54. Braganza, J.M., Wickens, D.G., Cawood, P., Dormandy, T.L.
Lipid peroxidation (free-radical-oxidation) products in bile from patients with pancreatic disease.
Lancet 1983a; ii: 375-8.

55. Braganza, J.M.
Pancreatic disease: A casualty of hepatic "detoxification".
Lancet 1983b; ii: 1000-2.

56. Braver, R.W., Leong, G.F., Holloway, R.J.
Mechanics of bile secretion: Effect of perfusion pressure and temperature on bile flow and bile secretion pressure.
Am. J. Physiol. 1954; 177: 103-12.

57. Brems, J., Ramirez, R., Kaminski, D.L.
Effect of 16, 16 Dimethyl prostaglandin E_2 on pancreatitis in the opossum.
Dig. Dis. Sci. 1983; 28: 928 (Abst).

58. Bub, H., Bürner, W., Riemann, J.F., Stolte, M.
Morphology of the pancreatic ductal epithelium after traumatization of the papilla of vater or endoscopic retrograde pancreatography with various contrast media in cats.
Scand. J. Gastroenterol. 1983; 18: 581-92.

59. Byrne, J.J., Berger, R.L.
The pathogenesis of acute cholecystitis.
Arch. Surg. 1960; 81: 812-26.

60. Camer, S.J., Tan, E.G.C., Warren, K.W., Braasch, J.W.
Pancreatic abscess. A critical analysis of 113 cases.
Am. J. Surg. 1975; 129: 426-31.

61. Carey, J.B.
Bile salt metabolism in man. The bile acids. Chemistry, Physiology and metabolism, Vol. 2. Physiology and metabolism (Hair PP, Kritcheusky, D. eds) New York, London, Plenum Press, 1973.

62. Carey, L.C.
Acute and chronic pancreatitis.
Surg. Clin. N. Am. 1975; 55: 325-38.

63. Caroli, J.
La radiomanometic biliare.
Sem. Hôp. Paris. 1946; 22: 1985.

64. Caroli, J.,Porcher, P., Pequignot, G., Delattre, M.
Contribution of cineradiography to study of the function of the human biliary tract.
Am. J. Dig. Dis. 1960; 5: 677-96.

65. Carr-Locke, D.L., Gregg, J.A.
Endoscopic manometry of pancreatic and biliary sphincter zones in man. Basal results in healthy volunteers.
Dig. Dis. Sci. 1981; 26: 7-15.

66. Carter, D.C.
Gallstone pancreatitis (editorial)
BMJ 1983a; 286: 1303-4.

67. Carter, D.C.
Biliary tract surgery in gallstone pancreatitis.
Hospital Update 1983b; 9: 879-93.

68. Case, R.M., Harper, A.A., Scratcherd, T.
The secretion of electrolytes and enzymes by the pancreas of the anaesthetized cat.
J. Physiol. 1969; 201: 335-48.

69. Case, R.M., Scratcherd, T.
On the permeability of the pancreatic duct membrane.
Biochim. et. Biophys. Acta. 1970; 219: 493-5.

70. Case, R.M.
Pancreatic secretion: cellular aspects.
In: Scientific Basis of Gastroenterology; Duthie, H.L., Wormsley, K.G. Eds, Edinburgh, Churchill Livingstone 1979; pp 163-198.

71. Chetlin, S.H., Elliott, D.W.
Biliary Bacteraemia.
Arch. Surg. 1971; 102: 303-7.

72. Chetty, U., Gilmour, H.M., Taylor, T.V.
Experimental acute pancreatitis in the rat: a new model.
Gut 1980; 21: 115-117.

73. Coelle, E.F., Adham, N., Elashoff, J., Lewin, K., Taylor, T.V.
Effect of prostaglandin and indomethacin on diet - induced acute pancreatitis in mice.
Gastroenterology 1983; 185: 1307-12.

74. Cox, J.L., Helfrich, L.R., Pass, H.I., Osterhaut, S.
The relationship between biliary tract infection and post-operative complications.
Surg. Gynecol. Obstet. 1978; 146: 233-6.

75. Creutzfeldt, W., Schmidt, H.
Aetiology and pathogenesis of pancreatitis (current concepts).
Scand. J. Gastroent. 1970; Suppl 6: 47-62.

76. Cruickshank, R.
Medical microbiology, 11th Edition.
Edinburgh, Churchill-Livingston, 1965, p. 1002.

77. Csendes, A., Kruse, A., Funch-Jensen, P., Oster, M.J., Ornsholt, J., Amdrup, E.
Pressure measurements in the biliary and pancreatic duct systems in controls and patients with gallstones, previous cholecystectomy, or common bile duct stones.
Gastroenterology 1979; 77: 1203-10.

78. Cushieri, A., Hughes, J.H.
Pancreatic reflux during operative choledochography.
Br. J. Surg. 1973; 60: 933-6.

79. Cushieri, A., Hughes, J.H., Cohen, M.
Biliary pressure studies during cholecystectomy.
Br. J. Surg. 1972; 59: 267-72.

80. Daniel, O.
The value of radiomanometry in bile duct surgery.
Ann. R.C.S. Eng. 1972; 51: 257-72.

81. Davenport, H.W.
Destruction of the gastric mucosal barrier by detergents and urea.
Gastroenterology 1968; 54: 179-85.

82. Davenport, H.W.
Effect of lysolecithin, digitonin and phospholipase A upon the dog's gastric mucosal barrier.
Gastroenterology 1970a; 59: 505-10.

83. Davenport, H.W.
Back diffusion of acid through the gastric mucosa and its physiological consequences.
In: Progress in gastroenterology, volume 2, chapter 3.
New York, Grune and Stratton, 1970b.

84. Davenport, H.W.
The gastric mucosal barrier.
Digestion 1972; 5: 162-5.

85. Davenport, H.W.
Physiology of the digestive tract.
Chicago, Year Book Medical Publishers, 1981.

86. De Bolla, A.R., Obeid, M.L.
Mortality in acute pancreatitis.
Ann. R.C.S. 1984; 66: 184-6.

87. De Fares, J.G., Sneddon, I.N.
An introduction to the mathematics of medicine and biology.
Amsterdam, North Holland Publishing Co., 1960; p. 342.

88. De Haas, G.H., Sarda, L., Roger, J.
Positional specific hydrolysis of phospholipids by pancreatic lipase.
Biochim. Biophys. Acta 1965; 106: 638-40.

89. Deliaris, P.G., Michail, P.O., Klonis, G.D. et al.
Biliary bacteriology based on operative bile culture.
Am. J. Gastroenterol. 1977; 68: 51-5.

90. Demol, P., Singer, M.V., Bernemann, D., Linzenhmeier, G., Goebell, H.
Excretion of azlocillin and mezlocillin by the normal pancreas and in acute pancreatitis in dogs and rats.
Digestion 1983; 27: 93-99.

91. Desnuelle, P., Figarella, C.
The normal pancreas, biochemistry.
In: The exocrine pancreas. Edited, H.T. Howat, H. Sarles.
London, Saunders, 1979.

92. Dimagno, E.P., Hendricks, J.C., Go, V.L.W., Dozois, R.R.
Relationships among canine fasting pancreatic and biliary secretions, pancreatic duct pressure, and duodenal phase II motor activity - Boldyreff revisited.
Dig. Dis. Sci. 1979; 24: 689-93.

93. Dimagno, E.P., Hendricks, J.C., Dozois, R.R., Go, V.L.W.
Effect of secretin on pancreatic duct pressure, resistance to pancreatic flow, and duodenal motor activity in the dog.
Dig. Dis. Sci. 1981; 26: 1-6.

94. Dineen, P.
The importance of the route of infection in experimental biliary tract obstruction.
Surg. Gynecol. Obstet. 1964; 119: 1001-8.

95. Doerr, W., Diezel, P.B., Grözinger, K.H., Lasch, H.G. et al.
Pathogenese der experimentellen autodigestiven pankreatitis.
Klin. Wschr. 1965; 43: 125-30.

96. Donahue, P.E., Akimoto, H., Ferguson, J.L., Nyhus, L.M.
Vasoactive drugs in acute pancreatitis.
Arch. Surgery 1984; 119: 477-80.

97. Doubilet, H., Colp, R.
Resistance of the sphincter of Oddi in the human.
Surg. Gynecol. Obstet. 1937; 64: 622-30.

98. Dragstedt, L.R., Haymond, H.E., Ellis, J.C.
Pathogenesis of acute pancreatitis (acute pancreatic necrosis).
Arch. Surg. 1934; 28: 232-91.

99. Dreiling, D.A., Druckman, L.J., Hollander, F.
The effect of complete vagisection and vagal stimulation on pancreatic secretion in man.
Gastroenterology 1952; 20: 578-86.

100. Dressel, T.D., Goodale, R.L., Hunninghake, D.B., Borner, J.W.
Sensitivity of the canine pancreatic intraductal pressure to subclinical reduction in cholinesterase activity.
Ann. Surg. 1979; 190: 6-12.

101. Duane, W.C., Wiegand, D.M.
Mechanism by which bile salt disrupts the gastric mucosal barrier in the dog.
J. Clin. Invest. 1980b; 66: 1044-9.

102. Duane, W.C., Wiegand, D.M., Gilbertsadt, M.L.
Intragastric duodenal lipids in the absence of a pyloric sphincter: quantitation, physical state, and injurious potential in the fasting and postprandial states.
Gastroenterology 1980a; 78: 1480-3.

103. Duprez, A., Godard, S., Platteborse, R.
La voie de dérivation intestielle et lymphatique de la sécretion exocrine du pancreas.
Bull. Acad. Roy. Med. Belg. 1968; 115: 118-27.

104. Durr, G.H.
Acute pancreatitis.
In: The exocrine pancreas; Howat and Sarles Eds.
London, W.B. Saunders Co. Ltd., 1979: pp 352-401.

105. Durst, A., Dishon, T., Rosenmann, Boss, J.H.
Factors in the pathogenesis of experimental acute pancreatitis in the rat.
Eur. Surg. Res. 1973; 3: 348-61.

106. Duval, M.K.
The effect of chronic pancreatitis on pressure tolerance in the human pancreatic duct.
Surgery 1958; 43: 798-801.

107. Dye, M., MacDonald, A., Smith, G.
The bacterial flora of the biliary tract and liver in man.
Br. J. Surg. 1978; 65: 285-7.

108. Eckhauser, F.E., Edgcomb, L.B., Knol, J.A., Stodel, W.E.
Experimental Acute Pancreatitis.
In: Pancreatic Disease, Diagnosis and Therapy; Dent, T.L., Eckhauser, F.E., Vinik, A.I., Turcotte, J.G. (Eds).
New York, Grune and Stratton, 1981: pp 189-203.

109. Edlund, Y., Ekholm, R., Zelander, T.
The effect of intraductal pressure increase on the ultrastructure of pancreas.
Acta. Chir. Scand. 1963; 125: 529-30.

110. Egdahl, R.H.
Mechanism of blood enzyme changes following the production of experimental pancreatitis.
Ann. Surg. 1958; 148: 389-99.

111. Elliott, D.W., Williams, R.D., Zollinger, R.M.
Alterations in pancreatic resistance to bile in the pathogenesis of acute pancreatitis.
Ann. Surg. 1957; 146: 669-76.

112. Elliott, D.W., Williams, R.D., Stewart, W.R.C.
The role of trypsin and of bile salts in the pathogenesis of acute pancreatitis.
Surg. Forum 1958; 9: 533-7.

113. Elliott, D.W.
Appraisal of the usefulness of various experimental models for the study of acute pancreatitis.
In: Beck, I.T., Sinclair, D.G.: The exocrine pancreas.
London, Churchill-Livingston, 1971, pp 86-102.

114. Elmslie, R.G. Magee, D.F., White, T.T.
Pancreatography with particular reference to activation of pancreatic enzymes.
Surg. Gynecol. Obstet. 1963; 117: 405-10.

115. Elmslie, R., White, T.T., Magee, D.F.
The significance of reflux of trypsin and bile in the pathogenesis of human pancreatitis.
Br. J. Surg. 1966; 53: 809-15.

116. Evander, A., Ihse, I., Lundquist, I.
Influence of anticholinergic treatment on rat pancreatic protein and amylase concentrations.
Scand. J. Gastroent. 1979; 14: 115-17.

117. Evander, A., Ihse, I., Lundquist, I.
Influence of hormonal stimulation by caerulein on acute experimental pancreatitis in the rat.
Eur. Surg. Res. 1981; 13: 257-68.

118. Evander, A., Hederstrom, E., Hultberg, B., Hise, I.
Exocrine pancreatic secretion in acute experimental pancreatitis.
Digestion 1982; 24: 159-67.

119. Evander, A., Lundquist, I., Ihse, I.
Influence of gastrointestinal hormones on the course of acute experimental pancreatitis.
Hepato-gastroenterol. 1982b; 29: 161-66.

120. Farber, E., Popper, H.
Production of acute pancreatitis with ethionine and its prevention by methionine.
Proc. Soc. Exp. Biol. Med. 1950; 74: 838-40.

121. Farquhar, M.G., Palade, G.E.
Junctional complexes in various epithelia.
J. Cell. Biol. 1963; 17: 375-84.

122. Ferrie, M.M., O'Hare, R., Joffe, S.N.
Acute and chronic pancreatitis in the rat caused by a closed duodenal loop.
Digestion 1978; 18: 280-5.

123. Fitz, R.H.
Acute pancreatitis: A consideration of pancreatic haemorrhagic suppurative and gangrenous pancreatitis and disseminated fat necrosis.
Boston. Med. Surg. J. 1889; 120: 181.

124. Flexner, S.
Experimental pancreatitis.
Johns Hopkins Hosp. Reports. 1901; 9: 743-71.

125. Flexner, S.
Constituent of bile causing pancreatitis and effects of colloids upon its action.
J. Exp. Med. 1906; 8: 167-77.

126. Forte, T.M., Silen, W., Forte, J.G.
Ultrastructural lesions in gastric mucosa exposed to deoxycholate: implications towards barrier concept.
In: Kasbekar, D.K., Sachs, G., Rehm, W.S. (Eds).
Gastric hydrogen ion secretion. Marcel Dokker Inc., New York; 1976: pp 1-28.

127. Foulis, A.K.
Histological evidence of initiating factors in acute necrotizing pancreatitis in man.
J. Clin. Pathol. 1980; 33: 1125-31.

128. Fox, J.N., Austin, J.L., Reber, H.A.
Effects of aspirin and pH on permeability of isolated perfused pancreatic duct.
Surgical Forum 1979; 30: 410-12.

129. Freund, H., Pfeffermann, R., Durst, A.L.
Gallstone pancreatitis: Exploration of the biliary system in acute and recurrent pancreatitis.
Arch. Surg. 1976; 111: 1106-8.

130. Frey, C.F., Lindenauer, S.M., Miller, T.A.
Pancreatic Abscess.
Surg. Gynaecol. Obstet. 1979; 149: 722-6.

131. Gambill, E.E.
Pancreatitis.
St. Louis, C.V. Mosby; 1973.

132. Gamklou, R., Edlund, Y.
Acute Alcoholic pancreatitis in the rat.
Scand. J. Gastoent. 1966; 1: 75-78.

133. Gedda, S., Van Der Linden, W.
What makes the peritoneal drain work? Pressure in the subhepatic space after biliary surgery.
Acta. Chir. Scand. 1983; 149: 703-6.

134. Geenen, J.E., Hogan, W.J., Shaffer, R.D., Stewart, E.T., Dodds, W.J., Arndorfer, R.C.
Endoscopic electrosurgical papillotomy and manometry in biliary tract disease.
JAMA 1977; 237: 2075-8.

135. Geokas, M.C., Murphy, D.R., McKenna, R.D.
The role of elastase in acute pancreatitis I. Intrapancreatic elastocolytic activity in bile-induced acute pancreatitis in dogs.
Arch. Path. 1968a; 86: 117-22.

136. Geokas, M.C., Rinderknecht, H., Wilding, P., Lillard, Y. et al.
Proteolytic enzymes in human pancreatic juice in acute pancreatitis.
J. Clin. Invest. 1968b; 47: 38-43.

137. Geokas, M.C., Rinderknecht, H., Swanson, V., Haverback, B.J.
Role of elastase in acute haemorrhagic pancreatitis.
Lab. Invest. 1969; 19: 235-9.

138. Geokas, M.C. Rinderknecht, H.
Free proteolytic enzymes in pancreatic juice of patients with acute pancreatitis.
Dig. Dis. 1974; 19: 591-8.

139. Geokas, M.C.
Lecture to Pancreatic Society of U.K.
Manchester, November 1983.

140. Gilsdorf, L., Urdaneta, L.F., Delaney, J.P., Leonard, A.S.
Central nervous system influences on pancreatic secretion, sphincteric mechanisms, and blood flow and their role in the course of pancreatitis.
Surgery 1967; 62: 581-8.

141. Goebell, H., Hotz, J.
Die Atiologie der akuten pancreatitis.
In: Forrell, M.M., ed. Handbuch der Inneren medizin, Band 3, Teil 6: Pankreas 5. Auflage. Berlin , Springer Verlag, 1979.

142. Gorbach, S.L., Tabaqchali, S.
Bacteria, bile, and the small bowel.
Gut 1969; 10: 963-72.

143. Goswitz, J.T.
Bacteria and biliary tract disease.
Am. J. Surg. 1974; 128: 644-6.

144. Graham, E.A., Peterman, M.G.
Further observations on the lymphatic origin of cholecystitis, choledochitis and the associated pancreatitis.
Arch. Surg. 1922; 4: 23-50.

145. Grant, D.A.W., Hermon-Taylor, J.
The purification of human enterokinase by affinity chromatography and immunoabsorption.
Biochem. J. 1976; 155: 243-54.

146. Grant, D.A.W., Hermon-Taylor, J.
Hydrolysis of artificial substrates by enterokinase and trypsin and the development of a sensitive specific assay for enterokinase in serum.
Biochem. Biophys. Acta. 1979; 567: 207-15.

147. Grant, D.A.W., Jones, P., Hermon-Taylor, J.
The biliary excretion of enterokinase in rats. Studies in normal, chronic ethanol-maintained and cirrhotic rats.
Biochem. J. 1981; 198: 315-9.

148. Grant, D.A.W., Terry, T.R., Hermon-Taylor, J.
Biliary excretion of enterokinase in rats: studies in alcoholic rats with fatty liver.
Gut 1983; 24: 16-19.

149. Greene, E.C.
Anatomy of the rat.
New York, Hafner Publishing Co., 1955, pp 101-5.

150. Greenwell, J.R.
The selective permeability of the pancreatic duct of the cat to monovalent ions.
Pflugers Arch. 1977; 367: 265-70.

151. Greuter, G., Widgren, S., Favre, H.
Lack of sensitivity and specificity of the renal clearance of amylase/clearance of insulin ratio in experimental acute rat pancreatitis with a study on the renal handling of amylase.
Eur. J. Clin. Invest. 1981; 11: 325-30.

152. Grossman, M.I.
Pancreatic secretion in the rat.
Am. J. Physiolol. 1958; 194: 535-9.

153. Grossman, M.I.
Experimental pancreatitis - Recent contributions.
JAMA 1959; 169: 1567-71.

154. Guelrud, M., Siegel, J.H.
Hypertensive pancreatic duct sphincter as a cause of pancreatitis.
Dig. Dis. Sci. 1984; 29: 225-31.

155. Hallenbeck, G.A.
Biliary and pancreatic intraductal pressures.
In: Handbook of Physiology; section 6: Alimentary canal, volume II secretion. Edited Code CF, Heidel, W.
American Physiological Society, Washington DC, 1967; 1007-25.

156. Halsted, W.S.
Retrojection of bile into the pancreas, a cause of acute haemorrhagic pancreatitis.
Bull. Johns Hopkins Hosp. 1901; 12: 179.

157. Ham, A.W., Cormack, D.W.
Histology, 8th Edition.
Philadelphia, J.B. Lippincott Co., 1979; pp 194-201.

158. Hammond, J.B., Mann, N.S.
Pancreatitis following the intraductal injection of partially purified enterokinase in dogs.
Dig. Dis. 1977; 22: 182-8.

159. Hand, B.H.
An anatomic study of the choledochoduodenal area.
Br. J. Surg. 1963; 50: 486-90.

160. Hansson, K., Lundh, G., Stenram, V.
Morphological and secretory studies in experimentally induced pancreatitis in rats.
Acta. Chir. Scand. 1961; 121: 274-8.

161. Hansson, K., Lundh, G., Stenram, V. et al.
Pancreatitis and free bile acids.
Acta Chir. Scand. 1963; 126: 338-45.

162. Hansson, K.
Experimental and clinical studies in aetiological role of bile reflux in acute pancreatitis.
Acta. Chir. Scand. 1967; suppl. 375; 1-102.

163. Harper, A.A.
Progress Report. The control of pancreatic secretion.
Gut. 1972; 13: 308.

164. Harvey, R.F.
Hormonal control of gastrointestinal motility.
Am. J. Dig. Dis. 1975; 20: 523-39.

165. Haslewood, G.A.D.
Bile Salts.
London, Methuen, 1968.

166. Haverback, B.J.
Trypsin, trypsinogen and trypsin inhibitor in human pancreatic juice.
Amer. J. Med. 1960; 29: 424-9.

167. Heinkel, K.
Die Ratte als versuchstier der experimentellen pancreas diagnostik.
Klin. Wochenschr. 1953; 31: 815-17.

168. Helenius, A., Simons, K.
Solubilization of membranes by detergents.
Biochem. Biophys. Acta. 1975; 415: 29-79.

169. Heller, F., Bouchier, I.A.D.
Cholesterol and bile salt studies on bile of patients with cholesterol gallstones.
Gut 1973; 14: 83-8.

170. Hendricks, J.C., Dimagno, E.P., Go, V.L.W., Dozois, R.R.
Reflux of duodenal contents into the pancreatic duct of dogs.
J. Lab. Clin. Med. 1980; 96: 912-21.

171. Hermann, R.E., Knowles, R.C.
Lethal factors and response to therapy in experimental bile-reflux pancreatitis.
Ann. Surg. 1965; 161: 456-65.

172. Hermann, R.E.
Manual of surgery of the gallbladder, bile and exocrine pancreas.
New York, Springer-Verlag, 1979.

173. Hermon-Taylor, J.
An aetiological and therapeutic review of acute pancreatitis.
Br. J. Hosp. Med. 1977a; 18: 546-9.

174. Hermon-Taylor, J., Perrin, J., Grant, D.A.W. et al.
Immunofluorescent localisation of enterokinase, in human small intestine.
Gut. 1977b; 18: 259-65.

175. Herring, P.T., Simpson, S.
The pressure of bile secretion and the mechanism of bile absorption in obstruction of the bile duct.
Proc. Roy. Soc. 1907; 79: 517-32.

176. Herriott, B.A., Palmer, A.A.
Enzyme changes in the plasma and peritoneal exudate of the rat following pancreatic duct obstruction.
Aust. J. Exp. Biol. Med. Sci. 1964; 42: 27-34.

177. Herriott, B.A. Sinnett, P.F., Palmer, A.A.
The occurence in the rat of an accessory pancreatic duct opening directly into the duodenum.
Aust. J. Exp. Biol. Med. Sci. 1965; 43: 175-79.

178. Herriott, B.A., Palmer, A.A.
Rupture of small ducts and acini in the pancreas of the rat and guinea pig following major duct obstruction.
Aust. J. Exp. Biol. Med. Sci. 1966; 44: 143-56.

179. Herva, P.
Experimental biliary pancreatitis in dogs.
Scand. J. Gastroent. (suppl. 8) 1970; 5: 1-63.

180. Heuman, R., Norrby, S., Sjödahl, R. et al.
Altered gallbladder bile composition in gallstone disease. Relation to gallbladder wall permeability.
Scand. J. Gastroent. 1980; 15: 581-6.

181. Hicken, N.F., McAllister, A.J.
Is the reflux of bile into the pancreatic ducts a normal or abnormal physiologic process?
Am. J. Surg. 1952; 83: 781-6.

182. Hoffman, A.F.
The enterohepatic circulation of bile acids in man.
Adv. Internal Med. 1976; 21: 501-34.

183. Hogan, W.J., Greenen, J.E., Schaffer, R.D., Dodds, W.J., Stewart, E.T., Arndorfer, R.C.
Sphincter of oddi pressure measurement in biliary tract disease before and after endoscopic sphincterotomy.
Gastroenterol. Endosc. 1977a; 23: 240-5.

184. Hogan, W.J., Dodds, W.J., Greenen, J.E., Schaffer, R.D., Stewart, E.T., Arndorfer, R.C.
Sphincter of oddi activity in man: a zone of unique phasic high-pressure contractions.
Edinburgh, Symposium on GI motility, paper No. 66, 1977b.

185. Hogan, W.J., Greenen, J.E., Dodds, W.J., Toouli, J., Venu, R., Helm, J.
Paradoxical motor response to cholecystokinin (CCK-OP) in patients with suspected spincter of oddi dysfunction.
Gastroenterology 1982; 82: 1085.

186. Holden, J.L., Berne, T.V., Rosoff, L.
Pancreatic abscess following acute pancreatitis.
Arch. Surg. 1976; III: 858-61.

187. Hollander, F..
The two-component mucous barrier.
Arch. Int. Med. 1954; 93: 107-20.

188. Hopton, D.S., Torrance, H.B.
Action of various new analgesic drugs on the human common bile duct.
Gut 1967; 8: 206-30.

189. Howard, J.M., Smith, A.K., Peters, J.J.
Acute pancreatitis: pathways of enzymes into the blood-stream.
Surgery 1949; 26: 161-70.

190. Howell, C.W., Bergh, G.S.
Pancreatic duct filling during cholangiography: its effect upon serum amylase.
Gastroenterology 1950; 6: 309-16.

191. Hunter, J.
On the digestion of the stomach after death.
Philosophical Transactions 1772; 62: 447-54.

192. Huttenen, R.,
Proteolytic enzymes in experimental rat pancreatitis.
Ann. Med. Exp. Biol. Fe. 1973; 51: 133-8.

193. Imrie, C.W.
Observations on acute pancreatitis.
Br. J. Surg. 1974; 61: 539.

194. Imrie, C.W., Benjamin, I.S., Ferguson, J.C. et al.
A single centre double blind trial of Trasylol therapy in primary acute pancreatitis.
Br. J. Surg. 1978; 65: 337.

195. Ivy, A.C., Gibbs, G.E.
Pancreatitis: A review.
Surgery, 1952; 31: 614-42.

196. Jackman, F.R., Hilson, G.R.E., Smith of Marlow, L.
Bile bacteria in patients with benign bile stricture.
Br. J. Surg. 1980; 67: 329-32.

197. Jacobs, M.L., Dagget, W.M., Civetta, J.M.
Acute pancreatitis: analysis of factors influencing survival.
Ann. Surg. 1977; 185: 43-51.

198. Jacobsson, B.
Determination of pressure in the common bile duct at and after operation.
Acta. Chir. Scand. 1957; 113_ 483-8.

199. Jalovaara, P., Apaja, M.
Alcohol and acute pancreatitis. An experimental study in the rat.
Scand. J. Gastroent. 1978; 13: 703-9.

200. Johnson, F.E., Reber, H.A., Wong, M.F., Carl, W.R.
Duodenal reflux into the pancreatic duct in dogs.
Surg. Forum. 1976; 27: 454-6.

201. Johnson, R.H., Doppman, J.
Duodenal reflux and the aetiology of pancreatitis.
Surgery 1967; 62: 462-7.

202. Kasugai, T., Kuno, N., Kizum. S.
Manometric endoscopic retrograde pancreatocholangiography.
Am. J. Dig. Dis 1974; 19: 485-502.

203. Keane, F.B., Dozois, R.R., Go, V.L.W., Dimagno, E.P.
Interdigestive canine pancreatic juice composition and pancreatic reflux and pancreatic sphincter anatomy.
Dig. Dis. Sci. 1981; 26: 577-84.

204. Keighley, M.R.B., Burdon, D.W.
Identification of bacteria in the bile by duodenal aspiration.
World J. Surg. 1978; 2: 255-9.

205. Keighley, M.R.B.
Infection and the biliary tree.
In: The Biliary Tract.
Edited. L.H. Blumgart.
Edinburgh, Churchill-Livingston 1982: pp219-35.

206. Keith, L.M., Barnes, J.E., Denkenalter, F.R.
Experimental study of interstitial injection trypsin and trypsinogen into the pancreas.
Arch. Surg. 1958; 77: 416-20.

207. Kellaway, I.W., Marriott, C.
The influence of some biological surfactants on the overturn time in goldfish.
J. Pharm. Pharmacol. 1977; 29: 508-9.

208. Kelly, T.R.
Gallstone pancreatitis: pathophysiology.
Surgery 1976; 80: 488-92.

209. Kelly, T.R.
Gallstone pancreatitis: The timing of surgery.
Surgery 1980; 88: 345-9.

210. Kelly, T.R.
Gallstone pancreatitis: The second time around.
Surgery 1982; 92: 571-5.

211. Kewenter, J., Kock, N.G., Lundberg, H.
Wound separation and intra-abdominal pressure.
Acta. Anaesth. Scand. 1969; 13: 97-102.

212. Keynes, W.M.
A non-pancreatic source of the proteolytic-enzyme amidase and bacteriology in experimental acute pancreatitis.
Ann. Surg. 1980; 191: 187-99.

213. Keynes, W.M.
Experimental acute pancreatitis.
In: The pancreas, (Ed) Keynes, W.M., Keith, R.G.
London, Heinemann, 1981, pp269-284.

214. Kivilaakso, E., Fromm, D., Silen, W.
Effect of lysolecithin on isolated gastric mucosa.
Surgery 1978; 84: 616-21.

215. Klein, E.S., Grateron, H., Rudick, J. Dreiling, D.A.
Pancreatic Intraductal pressure: I. A consideration of regulatory factors.
Am. J. Gastroent. 1983a; 78: 507-9.

216. Klein, E.S., Grateron, H., Toth, L., Dreiling, D.A.
Pancreatic Intraductal pressure: II. Effects of autonomic denervation.
Am. J. Gastroent. 1983b; 78: 510-12.

217. Kodama, T.
A light and electron microscopic study on the pancreatic ductal system.
Acta. Pathol. Jpn. 1983; 33: 297-321.

218. Kodesch, R., Dupont, H.L.
Infectious complications of acute pancreatitis.
Surg. Gynecol. Obstet. 1973; 136: 763-8.

219. Konok, G.P., Thompson, A.G.
Pancreatic ductal mucosa as a protective barrier in the pathogenesis of pancreatitis.
Am. J. Surg. 1969; 117: 18-23.

220. Kosterlitz, H.W.
Intrinsic and extrinsic nervous control of motility of the stomach and the intestines.
In: Handbook of Phsyiology, section 6: Alimentary Canal, vol. 4.
Edited by C.F. Code, W. Heidel, Washington D.C., American Physiological Society, 1968, pp 2147-71.

221. Kozoll, D.D., Dwyeer, G., Meyer, K.A.
Pathologic correlations of gallstones. A review of 1874 autopsies of patients with gallstones.
Arch. Surg. 1959; 79: 514-36.

222. Kraus, A.L.
Specimen collection, I Bile, J. Pancreatic juice.
In: The laboratory rat, volume II, research applications,
Baker, H.J., Lindsey, J.R., Weisbroth, S.H. (Eds).
New York, Academic press, 1980; pp 14-15.

223. Kraus, H., Kern, E.
Some current problems in biliary surgery.
Surg. Gynecol. Obstet. 1967; 124: 983.

224. Kümmerle, P., Hollander, L.F.
In: Acute pancreatitis. An interdisciplinary synopsis.
Eds. Hollander, L.F., Lehnert, P., Wanke, M.
Munich, Urban and Schwarzenberg, 1983. pp 41-42.

225. Kunitz, M., Northrop, J.H.
Isolation of a crystalline protein from pancreas and its conversion into a new crystalline proteolytic enzyme by trypsin.
Science 1933; 78: 558-62.

226. Lacy, E.R., Ito, S.
Microscopic analysis of ethanol damage to rat gastric mucosa after treatment with a prostaglandin.
Gastroenterology 1982; 83: 619-25.

227. Lambert, R.
Surgery of the digestive system in the rat.
Springfield, Thomas, 1965; pp. 107-170.

228. La Morte, W.W., Gaca, J.M., Wise, W.E., Birkett, D.H., Williams, L.F.
Choledochal sphincter relaxation in response to histamine in the primate.
J. Surg. Res. 1980; 28: 373-8.

229. Lampel, M., Kern, H.F.
Acute interstitial pancreatitis in the rat induced by excessive doses of a pancreatic secretoagogue.
Virch. Arch. A. 1977; 373: 97-117.

230. Lankisch, P.G., Winckler, K., Bokermann, M., Schmidt, H., Creutzfeldt, W.
The influence of glucagon on acute experimental pancreatitis in the rat.
Scand. J. Gastroent. 1974; 9: 725-9.

231. Lankisch, P.G., Koop, H., Winckler, K., Schmidt, H.
Continuous peritoneal dialysis as treatment of acute experimental pancreatitis in the rat. I. Effect on length and rate of survival.
Dig. Dis. Sci. 1979a; 24: 111-116.

232. Lankisch, P.G., Koop, H., Winckler, K., Schmidt, H.
Continuous peritoneal dialysis as treatment of acute experimental pancreatitis in the rat. II. Analysis of its beneficial effect.
Dig. Dis. Sci. 1979b; 24: 117-22.

233. Lankisch, P.G., Göke, B., Fölsch, U.R., Winckler, K., Otto, J., Creutzfeldt, W.
Influence of secretin on the course of acute experimental pancreatitis in rats.
Digestion 1983; 26: 187-91.

234. Lawson, H.H.
Effect of duodenal contents on the gastric mucosa under experimental conditions.
Lancet 1964; 1: 469-72.

235. Lennette, E.H., Balows, A., Hausler, W.J., Twant, J.P.
Manual of clinical microbiology. 3rd Ed. Washington D.C.
American Society of Microbiology. 1980. pp 83-375, 397-445.

236. Lötveil, T., Osnes, M., Aune, S.
Bacteriological studies of common bile duct bile in patients with gallstone disease and juxta-papillary duodenal diverticulae.
Scand. J. Gastroenterol. 1978; 13: 93-5.

237. Lou, M.A., Mandel, A.K., Alexander, J.L., Thadepalli, H.
Bacteriology of the human biliary tract and the duodenum.
Arch. Surg. 1977; 112: 965-7.

238. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.
Protein measurement with the Golin phenol reagent.
J. Biol. Chem. 1951; 193: 265-71.

239. Magee, A.I., Grant, D.A.W., Hermon-Taylor, J.
Further studies on the subunit structure and oligosaccharide moiety of human enterokinase.
Clin. Chim. Acta. 1981; 115: 241-54.

240. Mallet-Guy, P., Feroldi, J., Vidil, R.
L'éxploration fonctionelle manometrique et radiographique expérimentale du canal de wirsing : II Analyse des effets de l'hyperpression wirsungienne sur le parenchyme pancréatique.
Lyon Chir. 1958; 54: 321-42.

241. Manabe, T., Steer, M.L.
Protective effect of PGE_2 on diet induced acute pancreatitis in mice.
Gastroeneterology 1980; 78: 777-81.

242. Mann, F.C., Foster, J.P.
The secretory pressure of the liver with specific reference to the presence or absence of a gallbladder.
Am. J. Physiol. 1918; 47: 278-82.

243. Mann, F.C., Foster, J.P., Brimhall, S.D.
The relation of the common bile duct to the pancreatic duct in common domestic and laboratory animals.
J. Lab. Clin. Med. 1919; 5: 203-12.

244. Mann, F.C., Giordano, A.S.
The bile factor in pancreatitis.
Arch. Surg. 1923; 6: 1-30.

245. Mann, N.S.
Enterokinase induced pancreatitis.
Thesis. North Western University, Evanston, Ill., 1969.

246. Mann, N.S., Kadian, R.S., Narenderan, K.
Prevention of enterokinase induced acute pancreatitis with trasylol and soy bean trypsin inhibitor.
Gastroenterology 1977; 72: 1097-8.

247. Mann, S.K., Mann, N.S.
Effect of chlorophyll -a, fluorouracil, and pituitrin on experimental acute pancreatitis.
Arch. Pathol. Lab. Med. 1979; 103: 79-81.

248. Marriott, C., Martin, G.P., Newbery, R., Turner, N.C.
The effect of phophatidylcholine on bile-salt induced gastric ulceration in the rat.
J. Physiology 1984; 352: p. 72.

249. Martin, G.P., Marriott, C., Kellaway, I.W.
Direct effect of bile salts and phospholipids on the physical properties of mucus.
Gut. 1978; 19: 103-7.

250. Martin, G.P., Marriott, C.
Membrane damage by bile salts - the protective function of phospholipids.
J. Pharm. Pharmacol. 1981; 31: 754-9.

251. Mayer, A.D., McMahon, M.J.
Gallstone migration and early diagnosis of cholelithiasis in acute pancreatitis.
Gut 1983; 24: A988. (Abstract).

252. Mayer, A.D., McMahon, M.J., Benson, E.A., Axon, A.T.R.
Operations on the biliary tract in patients with acute pancreatitis : aims, indications and timing.
Ann. R.C.S. 1984; 66: 179-83.

253. Menguy, R.B., Hallenbeck, G.A., Bollmian, J.L., Grindlay, J.Y.
Intraductal pressures and spincteric resistance in canine pancreatic and biliary duct after various stimuli.
Surg. Gynecol. Obstet. 1958; 106: 306-20.

254. Mero, M.
Changing aetiology of acute pancreatitis.
Ann. Chir. Gynaecol. 1982; 71: 126-9.

255. Miller, T.A., Jacobson, E.D.
Gastrointestinal cytoprotection by prostaglandins.
Gut 1979; 20: 75-87.

256. Miller, T.A., Henagan, J.M.
Indomethacin decreases resistance of gastric barrier to disruption by alcohol.
Dig. Dis. Sci. 1984; 29: 141-149.

257. Mitchell, W.T., Stifel, R.E.
The pressure of bile secretion during chronic obstruction of the common bile duct.
Bull. Johns Hopkins Hosp. 1916; 27: 78-9.

258. Mizumoto, R., Kuratsuka, H., Kira, K., Honjo, I.
Pathogenesis of acute pancreatitis and changes of pancreatic ductal mucosa.
Am. J. Surg. 1971; 122: 398-401.

259. McCarthy, J.D.
Radiomanometry during biliary operations.
Arch. Surg. 1970; 100: 424.

260. McCutcheon, A.D.
Aetiological factors in pancreatitis.
Lancet 1962; i: 710-12.

261. McCutcheon, A.D., Race, D.
Experimental pancreatitis: Use of a new antiproteolytic substance; Trasylol.
Ann. Surg. 1963; 158: 233-9.

262. McCutcheon, A.D.
A fresh approach to the pathogenesis of pancreatitis.
Gut 1968; 9: 296-310.

263. McMahon, M.J., Shefta, J.R.
Physical characteristics of gallstones and the calibre of the cystic duct in patients with acute pancreatitis.
Br. J. Surg. 1980; 67: 6-9.

264. McMinn, R.M.H., Kuglee, J.H.
The glands of the bile and pancreatic ducts: autoradiographic and histochemical study.
J. Anat. 1961; 95: 1-26.

265. Moody, F.G., Zalewsky, C.A., Larsen, K.R.
Cytoprotection of the gastric epithelium.
World J. Surg. 1981; 5: 153-63.

266. Moqtaderi, F., Himal, H.S., Rudick, J., Dreiling, D.A.
Pancreatic transductal electrolyte flux.
Am. J. Gastroenterol. 1972; 58: 177-84.

267. Morris, G.P., Wallace, J.L., Harding, P.L. et al.
Correlations between changes in indicators of gastric mucosal barrier integrity at the time of exposure to "barrier breakers" and extent of haemorrhagic erosions one hour later.
Dig. Dis. Sci. 1984; 29: 6-11.

268. Mosley, J.G., Fox, J.N., Reber, H.A.
Aspirin secretion by the pancreas: Effect on the pancreatic duct mucosal barrier.
Surgery 1979; 86: 17-22.

269. Mosley, J.
The pancreatic duct mucosal barrier.
M.D. Thesis. University of Manchester , 1981.

270. M.R.C. Multicentre trial of glucagon and aprotinin.
Death from acute pancreatitis.
Lancet 1977; ii: 632-5.

271. Nevalainen, T.J., Seppä, A.
Acute pancreatitis caused by closed duodenal loop in the rat.
Scand. J. Gastroent. 1975; 10: 521-7.

272. Nevalainen, T.J.
The role of phospholipase A in acute pancreatitis.
Scand. J. Gastroent. 1980; 15: 641-50.

273. Ohlsson, K., Eddeland, A.
Release of proteolytic enzymes in bile-induced pancreatitis in dogs.
Gastroenterology 1975; 69: 668-75.

274. Ohnishi, H., Kosuzume, H., Ashida, Y., Kato, K., Honjo, I.
Effects of urinary trypsin inhibitor on pancreatic enzymes, and experimental acute pancreatitis.
Dig. Dis. Sci. 1984; 29: 26-32.

275. Olazabal, A., Nacimento, L.
Effect of indomethacin, aspirin, and fat-free diet on experimental pancreatitis in the rat.
J. Lab. Clin. Med. 1980; 96: 570-6.

276. Olazabal, A.
Deoxycholic acid alters electrolyte transport in the rat bile-pancreatic duct (Abst).
Gastroenterology 1981; 80: 1243.

277. Olazabal, A.
Effect of prostaglandins E_2 and I_2 and of indomethacin on deoxycholic acid - induced damage to the rat bile-pancreatic duct.
Gastroenterology 1983a; 84: 928-34.

278. Olazabal, A.
Prostacyclin treatment improves the survival in the deoxycholic acid model of pancreatitis in the rat.
Dig. Dis. Sci. 1983b; 28: 944 (Abst).

279. O'Leary, J.F., Droege, R., Gedgaudas, E., et al.
Measurement of pancreatic permeability by serial CT scan.
Surg. Gastroenterol. 1982; 1: 197-202.

280. O'Leary, J.F., Borner, J.W., Runge, W.J., et al.
Hyperplasia of pancreatic duct epithelium produced by exposure to sodium deoxycholate.
Am. J. Surg. 1984; 147: 72-77.

281. Opie, E.L.
The aetiology of acute haemorrhagic pancreatitis: Pathological report and experimental study.
Bull Johns Hopkins Hosp. 1901a; 12: 182-90.

282. Opie, E.L.
The relation of cholelithiasis to diseases of the pancreas and the fat necrosis.
Amer. J. Med. Sci. 1901b; 121: 27-30.

283. Opie, E.L.
Disease of the pancreas - its cause and nature.
Philadelphia: Lippincott, 1903.

284. Opie, E.L., Meakins, J.C.
Data concerning the etiology and pathology of haemorrhagic necrosis of the pancreas.
J. Exp. Med. 1909; 11: 561-78.

285. Orda, R., Hadas, N., Orda, S., Wiznitzer, T.
Experimental acute pancreatitis. Inducement by taurocholate sodium-trypsin injection into a temporary closed duodenal loop in the rat.
Arch. Surg. 1980; 115: 327-29.

286. Osborne, D.H., Imrie, C.W., Carter, D.C.
Biliary surgery in the same admission for gallstone - associated acute pancreatitis.
Br. J. Surg. 1981; 68: 758-61.

287. Osborne, D.H., Harris, N.W.S., Gilmour, H., Carter, D.C.
Operative cholangiography in gallstone associated acute pancreatitis.
J. R. C. S. Ed. 1983; 28: 96-100.

288. Owyang, C., Dozois, R.R., Dimagno, E.P., Go, V.L.W.
Relationships between fasting and postprandial pancreatico-duodenal pressures, pancreatic secretion, and duodenal volume flow in the dog.
Gastroenterolgoy 1977; 73: 1046-9.

289. Paloyan, D., Simonowitz, D., Skinner, D.B.
The timing of biliary tract operations in patients with pancreatitis associated with gallstones.
Surg. Gynecol. Obstet. 1975; 141: 737-9.

290. Papp, M., Breuer, J.H., Nemth, E.P., Fodor, I., Folly, G.
On the lysolecithin content of the pancreas in experimental acute pancreatitis.
Gastroenterology 1973; 65: 778-787.

291. Parks, D.A., Bulkley, G.B., Granger, D.N.
Role of oxygen-derived free radicals in digestive tract diseases.
Surgery 1983; 94: 415-21.

292. Parry, E.W., Hallenbeck, G.A., Grindlay, J.H.
Pressures in the pancreatic and common ducts: Values during fasting, after various meals and after spincterotomy; an experimental study.
Arch. Surg. 1955; 70: 757-65.

293. Pearson, K.
In: Regeneration of pancreas.
M.D. Thesis, University of Manchester 1974.

294. Pfeffer, R.B., Stasior, O., Hinton, J.W.
The clinical picture of sequential development of acute pancreatitis in the dog.
Surg. Forum 1957; 8: 248-51.

295. Pfeffer, R.B., Lazzarini-Robertson, A., Safadi, D., Mixter, G., Secoy, C.F., Hinton, J.W.
Gradations of pancreatitis, oedematous, through haemorrhagic experimentally induced by controlled injection of microspheres into blood vessels in dogs.
Surgery 1962; 51: 764-9.

296. Pirola, R.C., Davis, A.E.
Effect of pressure on the integrity of the duct-acinar system of the pancreas.
Gut. 1979; 11: 69-73.

297. Pissiotis, C.A., Condon, R.E., Nyhus, L.M.
Effect of vasopressin on pancreatic blood flow in acute haemorrhagic pancreatitis.
Am. J. Surg. 1972; 123: 203-8.

298. Poncelet, P.R., Thompson, A.G.
The role of clostridia in experimental pancreatitis.
Ann. R. Coll. Phys. Surg. Can. January 1969.

299. Poncelet, P., Thompson, A.G.
Action of bile phopholipids on the pancreas.
Am. J. Surg. 1972; 123: 196-202.

300. Poncelet, P.R., Thompson, A.G.
Role of infected bile in spasm of the sphincter of oddi.
Am. J. Surg. 1973; 126: 387-9.

301. Popper, H.L., Necheles, H.
Pathways of enzymes into the blood in acute damage of the pancreas.
Proc. Soc. Exp. Biol. (N.Y.) 1040; 43: 220-5.

302. Popper, H.L., Russell, K.C.
Transition of pancreatic oedema into pancreatic necrosis.
Surg. Gynecol. Obstet. 1948; 87: 79-85.

303. Potter, J.C., Mann, F.C.
Pressure changes in the biliary tract.
Am. J. Med. Sci. 1926; 171: 202-17.

304. Prince, M.,
Pancreatic apoplexy with a report of two cases.
Boston Med. Surg. J. 1882; 107: 28-32.

305. Ranson, J.H.C., Rifkind, K.M., Roses, D.F. et al.
Prognostic signs and the role of operative management in acute pancreatitis.
Surg. Gynecol. Obstet. 1974; 139: 69-74.

306. Ranson, J.H.C., Rifkind, K.M., Turner, J.W.
Prognostic signs and non operative peritoneal lavage in acute pancreatitis.
Surg. Gynecol. Obstet. 1976; 143: 209-15.

307. Ranson, J.H.C.
The timing of biliary surgery in acute pancreatitis.
Ann. Surg. 1979; 189: 654-662.

308. Rao, K.N., Tuma, J., Lombardi, B.
Acute haemorrhagic pancreatic necrosis in mice.
Gastroenterology 1976; 70: 720-6.

309. Rao, S.S., Watt. I.A., Donaldson, L.L., Crocket, A., Joffe, S.N.
A serial histological study of the development and progression of acute pancreatitis in the rat.
Am. J. Pathol. 1981; 103: 39-46.

310. Reber, H.A., Wolf, C.J., Lee, S.P.
Role of the main duct in pancreatic electrolyte secretion.
Surgical Forum 1969; 20: 382-4.

311. Reber, H.A., Roberts, C., Way, L.W.
"The pancreatic duct mucosal barrier".
Am. J. Surg. 1979a, 137: 128-34.

312. Reber, H.A., Mosley, J.G., Fox, J.N.
Effects of aspirin plus ethanol on the pancreatic duct mucosal barrier.
Surg. Forum. 1979b; 30: 414-16.

313. Reber, H.A., Mosley, J.G.
The effect of bile salts on the pancreatic duct mucosal barrier.
Br. J. Surg. 1980; 67: 59-62.

314. Reber, H.A., Tweedie, J.H., Adelstein, E.H.
Bile salt damage to the pancreatic duct: Ultrastructural changes and effect of 16, 16 dimethylprostaglandin E_2.
Gastroenterology 1981a; 80: 1258 (Abstr).

315. Reber, H.A., Tweedie, J.H.
Effects of a bile salt on the permeability of the pancreatic duct to macromolecules.
Surg. Forum. 1981b; 32: 219-21.

316. Reber, H.A., Farmer, R.C., Maslin, S.C.
Effects of bile on the permeability of the pancreatic duct to macromolecules.
Gastroenterology 1982; 82: 1156 (Abstr).

317. Reeman, F.C., Van Deenen, L.L.
The action of some synthetic lysolecithins and lecithins on erythrocytes and lipid bilayers.
Biochem. Biophys. Acta 1967; 137: 592-603.

318. Reinitz, E.R., Motoyama, E., Smith, G.J.W., Kerstein, N.D.
Pulmonary sequelae of experimental pancreatitis.
J. Surg. Res. 1977; 22: 566-579.

319. Ribiero, B.F., Cotton, P.B., Dicanari, J.B., Roberts, M., Laurence, B.
Duodenoscopic manometry of the bile duct, and spincter of oddi.
Gut. 1977; 18: 407-11.

320. Rich, A.R., Duff, G.L.
Pathogenesis of acute haemorrhagic pancreatitis.
Bull. Johns Hopkins Hosp. 1936; 58: 212-59.

321. Richards, C., Fitzgerald, P.J., Carol, B., Rosenstock, L., Lipkin, L.
Segmental division of the rat pancreas for experimental procedures.
Lab. Invest. 1964; 10: 1303-21.

322. Rittenbury, M.S., Hanback, L.D.
Snake antivenin. Effect on survival time in experimentally induced haemorrhagic pancreatitis.
Arch. Surg. 1969; 99: 179-84.

323. Robert, A.
Antisecretory, antiulcer, cytoprotective and diarrhoegenic properties of prostaglandins.
In: Advances in prostaglandin and thromboxane research.
Eds. Samuelsson, B., Paoletti, R., New York, Raven Press, 1976, vol 2, pp 507-20.

324. Robert, A.
Cytoprotection by prostaglandins.
Gastroenterology 1979; 77: 761-767.

325. Roberts, A., Nexamis, J.E., Field, S.O. et al.
16, 16 - Dimethyl - PGE_2 prevents caerulein - induced pancreatitis in rats.
Gastroenterology 1983; 84: 1287 (Abstr).

326. Roberts, P.F., Burns, J.
A histochemical study of mucus in normal and neoplastic human pancreatic tissue.
J. Pathol. 1972; 107: 87-94.

327. Robinson, T.M., Dunphy, J.E.
Continuous perfusion of bile protease activators through the pancreas.
JAMA 1963; 183: 53033.

328. Rohr, G., Keim, V., Burkhardt, R., Reschke, W.
Pancreatic duct permeability to exocrine pancreatic enzymes.
Digestion 1983; 28: 56-57.

329. Rosato, E.F., Cowan, R.P., Rosato, F.E.
Duodenal pressure as a factor in the cause of pancreatitis.
Surgery 1970; 68: 837-41.

330. Rosch, W., Koch, H., Demling, L.
Manometric studies during ERCP and endoscopic papillotomy.
Endoscopy 1976; 8: 30-3.

331. Rosenberg. I.H.
Influence of intestinal bacteria on bile acid metabolism and fat absorption.
Amer. J. Clin. Nutr. 1969; 22: 284-91.

332. Rosseland, A.R., Solhaug, J.H.
Early or delayed endoscopic papillotomy (EPT) in gallstone pancreatitis.
Ann. Surg. 1984; 199: 165-67.

333. Safrany, L., Cotton, P.B.
A preliminary report: urgent duodenoscopic sphincterotomy for acute gallstone pancreatitis.
Surgery 1981; 89: 424-8.

334. Sanfey, H., Bulkley, G.B. Cameron, J.L.
Pathogenesis of acute pancreatitis. Role of oxygen free radicals (unpublished paper).
:cited. Parks 1983, Surgery; 94: 415.

335. Sarosiek, J. Slomiany, B.L., Swierczek, J.
Effect of acetyl salicylic acid on the constituents of the gastric mucosal barrier.
Scand. J. Gastroenterol. 1984; 19: 150-3.

336. Schein, C.J., Beneventano, T.C.
Choledochal dynamics in man.
Surg. Gynecol. Obst. 1968; 27: 591-6.

337. Schein, C.J., Beneventano, T.C.
Biliary manometry: Its role in clinical surgery.
Surgery 1970; 67: 255-60.

338. Schiller, W.R., Suriyapa, C., Mutchler, J.H.W., Chen, S.C., Anderson, M.C.
Controlled ductal infusion and absorption from the interstitium of canine pancreas.
Arch. Surg. 1972; 105: 356-62.

339. Schiller, W.R., Suriyapa, C., Anderson, M.C.
A review of experimental pancreatitis.
J. Surg. Res. 1974; 16: 69-93.

340. Schmidt, H., Creutzfeldt, W.
The possible role of phospholipase A in the pathogenesis of acute pancreatitis.
Scand. J. Gastroent. 1969; 4: 39-48.

341. Schmidt, H., Creutzfeldt, W.
Etiology and pathogenesis of pancreatitis.
In: Gastroenterology (Ed.) Bockus H.L. 3rd Edition.
London, Saunders; 1976: pp 1005-19.

342. Schulenberg, C.A.R.
Operative cholangiography.
London: Butterworth, 1966.

343. Scott, A.J., Khan, G.A.
Origin of bacteria in bile duct bile.
Lancet 1967; 2: 790-2.

344. Scott, A.J.
Progress report. Bacteria and disease of the biliary tract.
Gut 1971; 12: 487-92.

345. Senn, N.
The surgery of the pancreas.
Trans. Am. Surg. Assoc. 1886; 4: 99-225.

346. Sewell, W.A., Young, J.A.
Secretion of electrolytes by the pancreas of the anaesthetized rat.
J. Physiol. 1975; 252: 379-96.

347. Shorter, R.G., Bollman, J.L., Baggenstoss.
Pressures in the common hepatic duct of the rat.
Proc. Soc. Exp. Biol. Med. 1959; 102: 682-4.

348. Shou, S.T., Gibson, J.B.
Experimental cholangitis and cholelithiasis.
Br. J. Exp. Pathol. 1968; 49: 563-73.

349. Silen, W.
"New concepts of the gastric mucosal barrier".
Am. J. Surg. 1977; 133: 8-12.

350. Silen, W., Merhav, A., Simson, J.N.L.
The pathophysiology of stress ulcer disease.
World J. Surg. 1981; 5: 165-74.

351. Simpson, C.J., Lewi, H.J.E., Carter, D.C.
Increase in perfusion pressure potentiates taurocholate-induced damage in the pancreatic duct.
Gut 1980; 21: A918 (Abst).

352. Simpson, C.J., Toner, P.G., Carr, K.E., Anderson, J.D., Carter, D.C.
Effect of bile salt perfusion and intraduct pressure on ionic flux and mucosal ultrastructure in the pancreatic duct of the cat.
Virchows Arch (cell pathol) 1983; 42: 327-42.

353. Singh, M., Webster, P.D.
Neurohormonal control of pancreatic secretion.
Gastroenterology 1978; 74: 294-309.

354. Sjödahl, R., Tagesson, C.
On the development of primary acute cholecystitis.
Scand. J. Gastroenterol. 1983; 18: 577-9.

355. Sjovall, J.
Bile acids in man under normal and pathological conditions.
Clin. Chim. Acta 1959; 5: 33-41.

356. Skillman, J.J., Silen, W.
Acute gastroduodenal stress ulceration: Barrier disruption of varied pathogenesis.
Gastroenterology 1970, 59: 478-81.

357. Skude, G., Wehlin, L., Maruyama, T., et al.
Hyperamylaseaemia and duodenoscopy and retrograde cholangiopancreatography.
Gut 1976; 17: 127-32.

358. Small, D.M.
Gallstones.
N. Eng. J. Med. 1968; 279: 588-93.

359. Smith, Lord of Marlow, Sherlock, S.
Surgery of the gallbladder and bile ducts. 2nd Edition.
London, Butterworths, 1981.

360. Standfield, N.J., Kakkar, V.V.
Prostaglandins and acute pancreatitis - experimental and clinical studies.
Br. J. Surg. 1983; 70: 573-6.

361. Steer, M.L.
Search for the Trigger Mechanism of Pancreatitis.
Gastroenterology 1984; 86: 764-6.

362. Stewart, E.T., Vennes, J., Geenen, J.E. (Eds)
Atlas of endoscopic retrograde cholangio-pancreatography.
St. Louis, C.V. Mosby, 1977.

363. Stone, H.H., Fabian, T.C., Dunlop, W.E.
Gallstone pancreatitis. Biliary tract pathology in relation to time of operation.
Ann. Surg. 1981; 194: 305-10.

364. Strack, A.A., Dreizin, D.H., Ketyer, S. et al.
Duodenal reflux in the genesis of acute pancreatitis.
Can. J. Surg. 1967; 10: 68-72.

365. Sum, P.T., Benscosme, S.A., Beck, I.T.
Pathogenesis of bile-induced acute pancreatitis in the dog.
Amer. J. Dig. Dis. 1970; 15: 637-45.

366. Suzuki, Y., Kobayash, A., Ohto, M., et al.
Bacteriological study of transhepatically aspirated bile.
Relation to cholangiographic findings in 295 patients.
Dig. Dis. Sci. 1984; 29: 109-15.

367. Takasugi, S., Yonezawa, H., Ikei, N., Kanno, T.
Prevention of acute experimental pancreatitis in rats and dogs by intraduodenal infusion of a synthetic trypsin inhibitor.
Digestion 1982; 24: 36-41.

368. Tanaka, M., Ikeda, S., Nakayama, F.
Non-operative measurement of pancreatic and common bile duct pressures with a microtransducer catheter and effects of duodenoscopic sphincterotomy.
Dig. Dis. Sci. 1981; 26: 545-52.

369. Taylor, T.V., Rimmer, S.
Pancreatic-duct reflux in patients with gallstone pancreatitis.
Lancet 1980; 1: 848-50.

370. Taylor, T.V., Torrance, H.B., Rimmer, S., Hillier, V., Lucas, S.B.
Operative cholangiography: Is there a statistical alternative.
Am. J. Surg. 1983; 145: 640-3.

371. Terano, A., Mach, T., Stachura, J., Tarnawski, A., Ivey, K.J.
Effect of 16, 16 dimethyl PGE_2 on aspirin induced damage to rat epithelial cells in tissue culture.
Gut 1984; 25: 19-25.

372. Terry, T.R., Grant, D.A.W., Hermon-Taylor, J.
Enterokinase-induced acute haemorrhagic pancreatitis in the rat.
Clin. Sci. 1982; 63: 59-63.

373. Terry, T.R., Grant, D.A.W., Hermon-Taylor, J.
Bacterial peptide aldehyde inhibitors in the chemotherapy of experimental acute necrotizing pancreatitis.
Br. J. Surg. 1983; 70: A38. p. 301.

374. Thal, A.P., Tansathithaya, P., Egner, W.
An experimental study of bacterial pancreatitis.
Surg. Gynecol. Obstet. 1956; 55: 353-61.

375. Thomas, J.M., Burcher, T.K., Baylis, C.R., Mortensen, N.J.
Serum amylase and pancreatic duct reflux during operative cholangiography.
Lancet 1983; ii; 1310 (letter).

376. Thurston, D.G.
Pathogenesis of gallstone pancreatitis.
Am. J. Surg. 1975; 129: 301-3.

377. Tiscornia, O.M., Martinez, J.L., Sarles, H.
Some aspects of human and canine macroscopic pancreas innervation.
Am. J. Gastroenterol. 1976; 55: 353-61.

378. Toouli, J. Greenen, J.E., Hogan, W.J., Dodds, W.J., Arndorfer, R.C.
Sphincter of oddi motor activity: A comparison between patients with common bile duct stones and controls.
Gastroenterology 1982a; 82: 111-17.

379. Toouli, J., Hogan, W.J., Geenen, J.E., Dodds, W.J., Arndorfer, R.C.
Action of cholecystokinin octapeptide on sphincter of oddi basal pressure and phasic wave activity in human.
Surgery 1982b; 92: 497-503.

380. Toouli, J., Dodds, W.J., Honda, R., Sarna, S., Hogan, W.J., Komorowski, R.A., Linehan, J.H., Arndorfer, R.C.
Motor function of the opossum sphincter of oddi.
J. Clin. Invest. 1983; 71: 208-20.

381. Toouli, J.
Sphincter of oddi motility.
Br. J. Surg. 1984; 71: 251-6.

382. Tormey, J.M., Diamond, J.M.
The ultrastructural route of fluid transport in rabbit gallbladder.
J. Gen. Physiol. 1967; 50: 2031-60.

383. Trapnell, J.E., Chir, T.M., Capper, W.M.
Trasylol in acute pancreatitis.
Am. J. Dig. Dis. 1967; 12: 409-12.

384. Trapnell, J.E., Duncan, E.H.L.
Patterns of incidence in acute pancreatitis.
B.M.J. 1975; ii: 179-83.

385. Tweedie, J.H., Mosley, J.G., Austin, J.L., Reber, H.A.
Effect of 16, 16 dimethyl prostaglandin E_2 on aspirin-induced permeability changes in the pancreatic duct.
Am. J. Surg. 1981; 141: 22-27.

386. Tykka, H., Mahlberg, K., Pantzar, P., Tailberg, T.
Phospholipase A_2 inhibitors and their possible clinical use in the treatment of acute pancreatitis.
Scand. J. Gastroent. 1980; 15: 519-28.

387. Uddin, K.K., Case, R.M., Garner, A.
Prostaglandin actions in the pancreas and biliary tract.
In: Mechanisms of mucosal protection in the upper gastrointestinal tract: Allen, A., Hemström, G., Garner, A., Silen, W., Turnberg, L.A. Editors. New York, Raven Press, 1984a; pp 303-10.

388. Uddin, K.K., Case, R.M.
Patterns of bicarbonate secretion in the upper gastrointestinal tract and their relation to gastric acid secretion.
In: Mechanisms of mucosal protection in the upper gastrointestinal tract.
Allen, A., Flemström, G., Garner, A., Silen, W., Turnberg, L.A. Editors. New York, Raven Press, 1984b; pp 129-34.

389. Valnes, K., Brandtzaeg, P., Elgjo, K., Stave, R.
Specific and non-specific humoral defence factors in the epithelium of normal and inflamed gastric mucosa.
Gastroenterology 1984; 86: 402-12.

390. Waldron, R.L.
Reflux pancreatography. An evaluation of contrast agents for studying the pancreas.
Am. J. Roentgen 1968; 104: 632-40.

391. Wanke, M.
Experimental acute pancreatitis.
Curr. Top. Pathol. 1970; 52: 64-142.

392. Wedgewood, K., Reber, H.A.
The effect of 16, 16 dimethyl prostaglandin E_2 on pancreatic duct permeability to macromolecules.
Gastroenterology 1984; 86: 1295 (Abst).

393. Weiner, S., Gramatica, L., Voegle, L.D. et al.
Role of the lymphatic system in the pathogenesis of inflammatory disease in the biliary tract and pancreas.
Am. J. Surg. 1970; 119: 55-61.

394. White, T.T., Magee, D.F.
Perfusion of the dog pancreas with bile without production of pancreatitis.
Ann. Surg. 1960; 151: 245-50.

395. White, T.T., Elsmlie, R.G., Magee, D.F.
Observations on the human intraductal pancreatic pressure.
Surg. Gynecol. Obstet. 1964; 118: 1043-45.

396. White, T.T.
Pancreatitis.
London: Arnold, 1966.

397. White, T.T., Murat, J.E., Morgan, A.
Pancreatitis I: review of 733 cases of pancreatitis from three Seattle Hospitals.
Northwest. Med. 1968; 67: 374-8.

398. White, T.T., Bourda, J.
A new observation on human intraductal pancreatic pressure.
Surg. Gynecol. Obstet. 1970; 130: 275-8.

399. White, T.T.
Inflammatory disease of the pancreas.
Advances in Surgery 1975; 247-304.

400. White, T.T., Bordley, J.
One per cent incidence of recurrent gallstones six to eight years after manometric cholangiography.
Ann. Surg. 1978; 188: 562-68.

401. Williams, L.F., Byrne, J.J.
The role of bacteria in haemorrhagic pancreatitis.
Surgery. 1968; 64: 967-72.

402. Williams, S.E., Turnberg, L.A.
Retardation of acid diffusion by pig gastric mucosa: a potential role in mucosal protection.
Gastroenterology 1980; 79: 299-304.

403. Wulsin, J.H., Silen, V.E.
Intraduct secretory pressure of pancreas in unanaesthetized dogs.
Surgery, 1953; 34: 9-19.

404. Yoshizawa, S.
Studies on pancreatic duct system.
Gastroenterol. Japonica. 1978; 13: 213-23.

405. Zieve, L., Vogel, W.C.
Measurement of lecithinase A in serum and other body fluids.
J. Lab. Clin. Med. 1961; 57: 586-90.